University of Alberta

Evaluation of Glucosamine Sulphate and Ibuprofen for Patients with Temporomandibular Degenerative Joint Disease with Pain

by

Norman M.R. Thie

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Medical Sciences -Oral Health Sciences

Edmonton, Alberta

Spring 2000

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-59887-X

Canada

University of Alberta

Library Release Form

Name of Author:	Norman M.R. Thie
Title of Thesis:	Evaluation of Glucosamine Sulphate and Ibuprofen for Patients with Temporomandibular Degenerative Joint Disease with Pain
Degree:	Master of Science
Year this Degree Granted:	2000

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

May 10/2000

Edmonton, Alberta, Canada.

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Evaluation of GS and Ibuprofen for Patients with Temporomandibular Degenerative Joint Disease with Pain by Norman M.R. Thie in partial fulfillment of the requirement of the requirement for the degree of Master of Science in Medical Sciences - Oral Health Sciences

Dr. PW Major (Supervisor)

Dr. F Jamali (Committee member)

Dr. WP Maksymowych (Committee member)

Dr. NG Prasad (Committee member)

Date: May 09/2000

ABSTRACT

The objective of this study was to compare the treatment potential of glucosamine sulphate (GS) and ibuprofen in patients diagnosed with temporomandibular joint (TMJ) osteoarthritis (OA). Fourty females and 5 males received either GS (500 mg tid) or ibuprofen (400 mg tid) for 90 days in a randomized double-blind study. Assessment - TMJ pain with function, pain free and voluntary maximum mouth opening, Brief Pain Inventory (BPI) questionnaire and masticatory muscle tenderness was performed after a one-week washout and at day 90. Acetaminophen (500 mg) dispensed for breakthrough pain, was counted every 30-days to day 120.

One hundred and seventy-six adults were interviewed, 45 (26 %) qualified, 39 (87%) completed the study (21 GS, 18 ibuprofen). Four discontinued due to stomach upset (3 ibuprofen, 1 GS), 1 due to dizziness (GS), 1 due to inadequate pain control (ibuprofen). Within group analysis revealed significant improvement compared to baseline of all variables in both treatment groups but no change in acetaminophen used. Fifteen (71%) and 11 (61%), GS and ibuprofen respectively, improved with positive clinical response set as 20% decrease in primary outcome (TMJ pain with function). Between group comparison revealed those patients taking GS had a significantly greater decrease in TMJ pain with function, impact of pain and acetaminophen used between day 90 and 120 compared with patients taking ibuprofen.

In conclusion, GS and ibuprofen reduce pain levels in patients with TMJ OA. Glucosamine sulphate had a significantly greater influence in reducing pain produced during function and impact of pain with daily activities. Glucosamine sulphate has a carry-over effect.

DEDICATION

This work is dedicated to all those who allow me to spread my creative wings, give me the freedom to be myself and push me to be the best that I can be.

ACKNOWLEDGEMENTS

I would like to acknowledge the following people and organizations for participating with this research:

My parents, Irmgard and Walter Thie for their love and continued support of my academic endeavors.

Dr. Paul William Major, supervisor, mentor and friend who kindly provided the wisdom and knowledge for shaping this thesis.

To my good friends and colleagues I have been so fortunate to have met while here in Edmonton: Anne and Brian Nebbe, Cynthia Blackman, Rena Perrotta, Elaine Corbett (&Cam), Bruce MacEachern (thanks for editing), Martin Parfitt, Mark Armstrong, Patricia Buchanan, Mitch Carr, Debbie and Don Gerhardt, Mike and Nan Kersey, Laurel and Kirby Cadman, Robin and Ian McKee, Cathy Biggs, Mauricio (soccer friend).
Carol Collinge ("you") for being a great friend over the last 2 years. May life bring you health and happiness.

The University of Alberta Fund for Dentistry, Edmonton Diagnostic Imaging Centre, Jamieson™, Apotex Inc.

Thanks to Will Leung at Strathcona Prescription Center for medication compounding.

The Staff of the Orofacial Pain Clinic, Roxanne Oliva and Kate Hoyano for keeping me on track when things get a little crazy and putting up with my sense of humor.

LIST OF TABLES

Table 1.1:	NSAID Classification	33
Table 1.2:	Pharmacokinetics of Selected NSAIDs	35
Table 2.1:	Exclusion Criteria	107
Table 2.2:	Within Treatment Group Comparison at Day 90	108
Table 2.3:	Between Treatment Group Comparison at Day 90 with Clinical Significance Set at 20% Improvement in Functional Pain	109
Table 2.4:	Percentage Functional Pain Improvement at Variable Clinical Levels of Significance	110
Table 3.1:	Potential Disease Modifying Osteoarthritic Drugs	118

LIST OF FIGURES

Figure 1.1: The Cyclooxygenase and Lipoxygenase Pathways of Arachidonic Acid Catabolism 37

Figure 1.2: The Role of Cyclooxygenase Isoforms 1 and 2 in Normal and Inflamed Tissues 39

LIST OF ABBREVIATIONS

CAS	Colour analogue scale
CNS	Central nervous system
COX	Cyclooxygenase
CS	Chondroitin sulphate
DG	D-glucosamine
DJD	Degenerative joint disease
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
GAG	Glycosaminoglycan
GI	Gastrointestinal
GS	Glucosamine sulphate
i.a.	Intraarticular
i.m.	Intramuscular
i.v.	Intravenous
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IL-1	Interleukin-1
MMP	Matrix metalloproteases
MRI	Magnetic Resonance Imaging
NO	Nitric oxide
NSAID	Nonsteroidal antiinflammatory drug
OA	Osteoarthritis
PA	Plasminogen activator

PG	Prostaglandin
PPT	Pressure Pain Threshold
TENS	Transcutaneous nerve stimulation
TGF	Transforming growth factor
tid	Three times per day
TMD	Temporomandibular disorder
TMJ	Temporomandibular joint
TNF	Tumor necrosis factor
VAS	Visual analogue scale
WOMAC	Western Ontario and McMaster University Osteoarthritis Index

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW 1

1.1 GENERAL INTRODUCTION *1*
1.2 STATEMENT OF PROBLEM *2*
1.3 RESEARCH QUESTIONS *5*
1.4 HYPOTHESES *5*
1.4.1 Null hypotheses 5
1.4.2 Alternate hypotheses 6
1.5 LITERATURE REVIEW *6*
1.5.1 Overview of Histological Features of OA in Hyaline Lined Synovial Joints *6*
1.5.1.1 Articular Cartilage 7
1.5.1.2 Subchondral Bone 9
1.5.1.3 Synovial Membrane 10
1.5.2 Overview of Biochemical Features of OA in Hyaline Lined Synovial Joints *10*
1.5.3 Temporomandibular Degenerative Joint Disease *13*
1.5.3.1 Introduction 13
1.5.3.2 Epidemiology 14
1.5.3.3 Clinical Features 14
1.5.3.4 Radiographic Features 15
1.5.3.5 Pathogenesis of TM Degenerative Joint Disease 16
1.5.3.5.1 Aging 16
1.5.3.5.2 Occlusion 17
1.5.3.5.3 Joint Loading 17
1.5.3.5.4 Disc Displacement 18
1.5.3.6 Articular Cartilage - Structure and Chemistry 20
1.5.3.7 Histology and Biochemistry of Temporomandibular DJD 24
1.5.3.7.1 Articular Cartilage 25
1.5.3.7.2 Synovial Membrane 26
1.5.3.7.3 Subchondral Bone 27
1.5.3.8 Proposed Mechanisms of TMJ DJD 27
1.5.3.9 Estrogen and DJD 29
1.5.3.10 Overview of Treatment of TMJ DJD 30
1.5.4 Nonsteroidal Antiinflammatories *32*
1.5.4.1 Historical Overview 32
1.5.4.2 Classification 32
1.5.4.3 Pharmacokinetics 34
1.5.4.4 Pharmacodynamics 36
1.5.4.5 Central Nervous System Effects 40
1.5.4.6 NSAID's and Osteoarthritis 41
1.5.4.6.1 Side Effects 42
1.5.4.6.2 Cyclooxygenase1 and 2 43

1.5.4.6.3 Effects on Articular Cartilage 43
1.5.5 Glucosamine Sulphate *45*
1.5.5.1 Physical Properties and Pharmacokinetics: 45
1.5.5.2 Pharmacodynamics 46
1.5.5.3 Glucosamine Sulphate for Osteoarthritis 47
1.5.6 Chondroitin Sulphate *49*
1.6 References *53*

CHAPTER 2

EVALUATION OF GLUCOSAMINE SULPHATE AND IBUPROFEN FOR TREATMENT OF TEMPOROMANDIBULAR OSTEOARTHRITIS: A RANDOMIZED DOUBLE BLIND CONTROLLED 3-MONTH CLINICALTRIAL 85

2.1 INTRODUCTION *85*
2.2 MATERIALS AND METHODS *87*
2.3 RESULTS *90*
2.4 DISCUSSION *91*
2.5 REFERENCES *99*

CHAPTER 3

GENERAL DISCUSSION 111

3.1 INTRODUCTION *111*
3.2 STUDY LIMITATIONS *115*
3.3 CLINICAL IMPLICATIONS *116*
3.4 DIRECTIONS FOR FUTURE STUDIES *117*
3.5 REFERENCES *119*

APPENDICES 125

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis[1,2]. Clinically, it is characterized by joint pain, tenderness, limitation of movement, crepitus, effusions and variable degrees of local inflammation, but without systemic effects. Pathologically degenerative changes of articular cartilage, sclerosis of subchondral bone, subchondral bone cysts, marginal growth of bone and cartilage (osteophytes) and synovial inflammation characterize OA. A recent definition of OA gives emphasis to the concept that OA may not represent a single disease entity:[3]

Osteoarthritis is a group of overlapping distinct diseases, which may have different etiologies but with similar biologic, morphologic and clinical outcomes. The disease processes not only affect the articular cartilage but also involve the entire joint, including the subchondral bone, ligaments, capsule, synovial membrane, and periarticular muscles. Ultimately, the articular cartilage degenerates with fibrillation, fissures, ulceration, and full thickness loss of the joint surface.

A classification of OA into two major groups was developed at the 'Workshop on Etiopathogenesis of Osteoarthritis'[4]. The first is idiopathic (sometimes referred to as primary), that is divided into two forms: localized or generalized, the latter representing forms involving 3 or more joint groups[5]. Idiopathic OA (the more common type) is considered OA in which no single definable precipitating event or disease has been identified as a causative factor. The other group of OA is classified as secondary OA. Secondary OA is associated with some clearly defined event (e.g. trauma) or pathology. Secondary OA has been further subdivided by the specific cause or associated disease[4].

Within the literature there are many synonymous terms for OA, including osteoarthrosis and DJD. The suffix "itis" means inflammation, however, inflammation is not considered the primary event in OA. For this reason the terms osteoarthrosis and DJD may be preferred to describe the degenerative and biomechanical characteristics of OA[6]. Today there is still no universal consensus regarding the terminology of OA. The terms DJD and OA are used interchangeably in this thesis to describe the overall disease process whether or not inflammation is considered to be the primary event in the process.

The readers of this thesis should also note that the term "cartilage" is used as a general descriptor only, describing the articular tissue of synovial joints. A distinction needs to be made between synovial joints in general and the temporomandibular joint (TMJ) since the articular tissue of the TM condyle is a dense fibrous connective tissue not cartilage. The term fibrocartilage has appeared in the literature to describe the TM articular tissue since it contains a small percentage of chondrocytes, the cells of cartilagenous joints. The term fibrocartilage is used in this thesis to denote the tissue covering the TM condyle and articular eminence.

1.2 STATEMENT OF PROBLEM

Osteoarthritis brings discomfort and disability to millions of North Americans each year and the costs involved in treating OA are expected to reach 1% of the United States gross national product in year 2000[7]. A recent report in the United States on resource utilization and cost of care for 10,101 OA patients, revealed that the average annual cost for treating this disorder is $543.00 per person[8]. This figure seems unremarkable against $2,162.00 per person spent annually on rheumatoid arthritis patients, however,

prevalence of OA is far greater making OA seven times more costly to the managed care provider[8].

Whereas rheumatoid arthritis is a chronic systemic inflammatory disease affecting many joints, OA can be limited to one or very few joints. It is not uncommon to know a friend or family member diagnosed with OA of either the knee, hip or hand joints for example. Osteoarthritis is a degenerative disease of joints characterized by a progressive loss of normal structure and function of articular cartilage. Its pathogenesis, although having been correlated to joint use, age and "wear and tear", remains uncertain.

The TMJ is not immune in development of OA, or more commonly referred to as degenerative joint disease (DJD) of the TMJ. A recent review by Kamelchuk and Major[9], reported approximately 8% to 12% of patients seeking treatment at temporomandibular dysfunction clinics receive a diagnoses of DJD. Once the diagnosis is made, and pain is an issue, the clinician generally places the patient on a soft diet, advises jaw functioning within a pain-free range, and prescribes a nonsteroidal anti-inflammatory drug (NSAID)[9]. Nonsteroidal anti-inflammatories such as ibuprofen have traditionally been the medicines of first choice[10,11].

Nonsteroidal anti-inflammatory drugs have a well-documented record of relieving pain and reducing inflammation, but this record is not completely favorable. Unfortunately many of these medications are known to cause multiple side effects, notably upper gastrointestinal (GI) damage[12]. Geis *et al*[13] reported that 14.6% to 43.9% of OA patients treated with traditional NSAID's develop gastric ulcers after 6 months of therapy. Epidemiological and clinical studies report that the cost of NSAID treatment should be multiplied by a coefficient range of 1.36 to 3 when the cost of treating the induced GI damage is also taken into account[14].

There is now a growing body of evidence that many of the more traditional NSAID's exacerbate the loss of the articular cartilage necessary for joint health by inhibiting proteoglycan synthesis at the level of the chondrocyte[15-19]. This problem has prompted research into medicinal agents that have cartilage sparing, regenerative capacities and pain relieving effects.

Glucosamine is a naturally occurring aminomonosaccaride in the human body, biosynthesized from glucose and used to form glycosaminoglycans (GAG's), a constituent of proteoglycans which is an important component of the extracellular matrix (ECM) of articular cartilage[20]. It's potential as a therapeutic agent for OA was first reported in 1969[21]. Investigations in the early 1980's found those patients with OA of the knee, when administered glucosamine compared to placebo, reported gradual and progressive reduction of articular pain and tenderness and improvement in the range of motion[22-25]. Oral administration of glucosamine sulphate (GS) has also been reported to not irritate the GI tract[26] and may stimulate the production of protective gastric mucoproteins[27]. Several studies have also reported that therapeutic benefits of GS were maintained for weeks after therapy was discontinued[28,29].

Glucosamine sulphate is regarded as a food supplement and is available in health food and drug stores. It's potential as an adjunctive medicine for OA is gaining growing acceptance, supported by tissue, animal and human studies. Like other joints of the body, traditional pharmacological methods for treating patients with degenerative disease of the TMJ have largely depended on NSAID's. "Natural medicines" like GS may also provide pain relief for this patient population without the inherent side effects of many traditional NSAID's. To date there are no published clinical trials to assess the efficacy of GS in treatment of DJD of the TMJ. The articular surface of the TMJ has structural differences and direct comparison to other synovial joints with hyaline cartilaginous articular surfaces may not be appropriate. It is therefore the intent of this

study to investigate the potential GS for treating patients diagnosed with TMJ DJD with associated pain.

1.3 RESEARCH QUESTIONS

1. Is GS more effective than ibuprofen in reducing TMJ functional pain (pain on yawning, pain on chewing, pain on talking, pain on laughing)?
2. Is GS more effective than ibuprofen for increasing pain free and voluntary range of jaw opening?
3. Is GS more effective than ibuprofen for reducing pain intensity (pain at it's worst, pain at it's least, pain on average, pain right now) and pain impact in terms of how much it interferes with general activity, mood, walking ability, normal work, relations with other people, sleep, enjoyment of life?
4. Is GS more effective than ibuprofen for reducing secondary masticatory muscle pain?
5. Will patients who have taken GS use less acetaminophen for breakthrough pain than those patients taking ibuprofen?

1.4 HYPOTHESES

1.4.1 Null hypotheses

1. Glucosamine sulphate is not more effective than ibuprofen in reducing functional pain (pain on yawning, pain on chewing, pain on talking, pain on laughing).
2. Glucosamine sulphate is not more effective than ibuprofen for increasing pain free and voluntary range of jaw opening.
3. Glucosamine sulphate is not more effective than ibuprofen for reducing pain intensity (pain at it's worst, pain at it's least, pain on average, pain right now) and pain impact in terms of how much it interferes with general activity, mood, walking ability, normal work, relations with other people, sleep, enjoyment of life.

4. Glucosamine sulphate is not more effective than ibuprofen for reducing secondary masticatory muscle pain.
5. Patients taking GS will not use less acetaminophen for breakthrough pain than those patients taking ibuprofen.

1.4.2 Alternate hypotheses

1. Glucosamine sulphate is more effective than ibuprofen in reducing functional pain (pain on yawning, pain on chewing, pain on talking, pain on laughing).
2. Glucosamine sulphate is more effective than ibuprofen for increasing pain free and voluntary range of motion.
3. Glucosamine sulphate is more effective than ibuprofen for reducing pain intensity (pain at it's worst, pain at it's least, pain on average, pain right now) and pain impact in terms of how much it interferes with general activity, mood, walking ability, normal work, relations with other people, sleep, enjoyment of life.
4. Glucosamine sulphate is more effective than ibuprofen for reducing secondary masticatory muscle pain.
5. Patients taking GS will use less acetaminophen for breakthrough pain than those patients taking ibuprofen.

1.5 LITERATURE REVIEW

1.5.1 Overview of Histological Features of OA in Hyaline Lined Synovial Joints

Osteoarthritis typically affects all of the tissues that form synovial joints. Primary changes consist of loss of articular cartilage, remodelling of subchondral bone and formation of osteophytes. These changes are overviewed in the following sections.

1.5.1.1 Articular Cartilage

Many of the mechanisms involved in the degeneration of articular cartilage in OA remain unknown, but three overlapping stages can be described[30]:

1) Initial stage in which disruption or alteration of articular cartilage matrix and increased water characterize this stage. Features of this stage include decreases in the aggregation of proteoglycans, the concentration of aggrecans and the length of GAG chains. There is no change in the concentration of Type II collagen but there are alterations in the collagenous framework that includes changes in the relationships between the minor collagen's and collagen fibrils. Cartilage degeneration frequently begins with the disruption of collagen fibrils in the superficial zone. These changes may allow the aggrecan molecules to swell.

   Overall the changes seen in this stage are thought to increase the ease with which water and other molecules move through the matrix and decrease the stiffness of the matrix thereby making the tissue vulnerable to mechanical damage.

2) Second stage: chondrocytic response to tissue damage.

   As a response to the disruption of their matrix, chondrocytes release mediators that stimulate a cellular response. The response consists of increased matrix synthesis as well as degradation and chondrocyte proliferation. Clusters or clones of proliferating cells surrounded by newly synthesized matrix molecules are a histologic hallmark of the chondrocytic response to the degeneration of cartilage. The increased synthesis of matrix macromolecules, and to a lesser extent cell proliferation, counters the catabolic processes and may stabilize and/or restore the tissue. This repair response may continue for years. Cartilage superficial tearing of the articular tissue occurs along more or less vertical lines; clefts and fissures are thereby generated and the cartilage becomes fibrillated. Fibrillation, if not repaired,

gives rise to extensive fragmentation of the articular cartilage and eventually to subchondral bone.

3) Third stage: decline in the chondrocytic response to maintain the articular cartilage resulting in progressive loss of cartilage with time.

   Advanced stages of OA are characterized by complete loss of articular cartilage (denudation of the subchondral bone). Chondrocyte necrosis is apparent[31]. The collagen network is severely disorganized and disintegrated, and histochemical stains for proteoglycans show severe depletion of proteoglycans[31].

It is worth pointing out that when articular cartilage is damaged the defects are classified as either partial (superficial) or full-thickness[32]. In tissues such as skin or bone, injury elicits an inflammatory response followed by repair. Lacerations and fractures become filled with a fibrin clot and inflammatory cells. Mesenchymal cells migrate into the clot, proliferate and differentiate, and the lesion becomes plugged with dense scar or native tissue[33]. In early OA partial thickness defects are common but do not penetrate through the zone of calcified cartilage into the subchondral bone. These defects have no access to the inflammatory cells, macrophages and mesenchymal stem cells located within the marrow. Therefore no fibrin clot develops within the clefts and fissures or fibrillated cartilage, and neither chondrocytes nor mesenchymal cells migrate into these regions[32,34]. Consequently, they do not become filled with repair tissue. On the other hand with full-thickness defects or advanced degeneration of articular cartilage, penetration of subchondral bone disrupts subchondral blood vessels. Blood from the marrow wells up into the lesion leading to the formation of a space-filling fibrin clot containing inflammatory cells and undifferentiated mesenchymal cells that differentiate into cells with the morphological features of chondrocytes[35-38]. The full thickness defects become filled with a fibrocartilagenous repair tissue.

The fibrocartilagenous repair tissue has been shown to contain Type I collagen, the type found within TMJ articular tissues. Human cartilage explants from OA joints were reported in 1973 to synthesize collagen Type I as well as collagen Type II in contrast to explants from normal joints that synthesize only collagen Type II[39]. Immunofluorescence studies have shown chondrocytes surrounded by collagen Type I in articular cartilage from OA joints[40,41]. Osteophytes and reparative fibrocartilage also express collagen Type I[41-43].

1.5.1.2 Subchondral Bone

Subchondral bone is thought to play an important role in OA, but whether the bony changes observed during OA initiates or is involved in the progression of cartilage loss remains a matter of debate. Experiments with rabbit knee joints have found that repetitive impulse loading results in early bony responses characterized by increased bone formation and stiffening followed by articular cartilage degeneration[44]. Subchondral bone changes have also been proposed as an early event in the pathogenesis of OA in guinea pig femoral heads[45]. Radin and colleagues[46] believe sclerotic changes in subchondral bone predispose to cartilage matrix degeneration. In contrast, longitudinal studies of subchondral bone responses in dogs[47] have found that bony changes were associated with progression of cartilage degeneration, rather than initial development.

Regardless of initiation, the alterations of the subchondral bone that occur with OA include increased density (subchondral sclerosis), formation of cyst-like bone cavities containing myxoid, fibrous or cartilagenous tissue and the appearance of regenerating cartilage within and/or on the subchondral bone surface[30]. The increased density of the subchondral bone resulting from formation of new layers of bone on existing trabeculae is considered the first sign of DJD in subchondral bone[30]. At the end stage of the OA disease process, the articular cartilage is completely lost, leaving

thickened subchondral bone articulating with a similarly denuded osseous surface[30]. The bone remodelling combined with a loss of articular cartilage changes the shape of the joint and can lead to shortening of the involved limb, deformity and instability[30].

1.5.1.3 Synovial Membrane

Although, by definition, OA is not considered to be a prominent inflammatory condition, some degree of synovial hypertrophy and fibrosis is seen in the majority of symptomatic cases[48]. The loss of articular cartilage leads to secondary changes in synovial tissue with mild to moderate inflammatory reaction and the tissue may contain fragments of articular cartilage[49]. Synovial macrophages and fibroblasts are stimulated, resulting in generation of a broad range of inflammatory mediators, resembling those found in inflammatory joint disease such as rheumatoid arthritis[48]. These biochemical mediators involved in OA are discussed in the following section.

1.5.2 Overview of Biochemical Features of OA in Hyaline Lined Synovial Joints

The previous section described initial changes in osteoarthritic cartilage that include increased water content, loss of proteoglycans and alterations in the collagenous framework. This also consists of a cellular response by the chondrocytes and chondrocyte proliferation into clusters or clones with newly synthesized ECM components. This histologically observed chondrocytic repair response is mediated by growth factors, with insulin-like growth factor (IGF-1) considered to be the most important[50]. Insulin-like growth factor-1 stimulates chondrocyte proteoglycan synthesis and inhibits proteoglycan catabolism[50]. Nitric oxide (NO) may also play a role in this chondrocytic response, since chondrocytes produce this molecule in response to a variety of mechanical and chemical stresses[51,52]. Other growth factors produced locally by chondrocytes and neighboring tissues (synovial membrane and bone) affect cartilage

metabolism and include fibroblast growth factor, epidermal growth factor, platelet-derived growth factor and transforming growth factor-β (TGF-β)[53]. A variety of cytokines and protease's are also important in normal tissue turnover and degradation of ECM components. There is a balance established between repair and degradation of the ECM components. Unfortunately if this balance fails, chondrocytes start secreting more proteolytic enzymes and catabolic cytokines. The factors that cause the balance to tip remains unknown but could include: alteration in the cartilage macromolecule structure, chondrocyte phenotypic change, interleukin-1β (IL-1β) overproduction, increase susceptibility of chondrocytes to cytokine-induced degradation, focal over-expression of IL-1β and tumor necrosis factor-α (TNF-α) receptors, growth factor synthesis and bioactivity down-regulation, reduction of chondrocyte sensitivity to growth factors and chondrocyte apoptosis[54-57].

Proteolytic enzymes are involved in normal tissue turnover and degradation of ECM components. A great deal of attention has been focused on identifying the protease responsible for initial occurrence of ECM digestion. Current literature has focussed on the matrix metalloproteases (MMP). Three groups are elevated in OA: the collagenases, stromelysins and gelatinases[58]. A recently discovered membrane type MMP may also be important[58]. The synthesis of these enzymes by chondrocytes, synovial cells, and inflammatory cells is mediated by a variety of cytokines, growth factors and hormones in normal articular cartilage including tissue inhibitor of metalloprotease and plasminogen activator (PA) inhibitor[58]. An imbalance between protease and protease inhibitor levels may tip the balance in the favour of OA disease progression.

The integrity of articular tissue is thought to be maintained through a balance in cytokine-driven anabolic and catabolic processes[59]. At the clinical stage of the disease,

changes caused by OA involve cartilage and the synovial membrane where an inflammatory reaction (synovitis) is often seen. Synovial inflammation is of fundamental importance in OA mainly due to the secretion of mediators such as the cytokines[60]. Cytokines induce the synthesis of protease's that results in increased ECM degradation and proteoglycan depletion. Interleukin-1β and TNF-α are considered to be the most involved in the catabolic process of OA[60]. They are produced by the synovial membrane, diffuse into the cartilage through the synovial fluid and activate the chondrocytes to produce proinflammatory cytokines[58].

Other cytokines expressed in synovial membrane or fluid of OA patients include IL-6, IL-7, IL-8 and leukemia inhibitory factor[59]. All are upregulated by the proinflammatory cytokines IL-1β and TNF-α, and also have the ability to increase the synthesis of the latter two[59]. Their exact roles in OA have not been clearly established.

Nitric oxide may promote cartilage catabolism in OA. Compared to normal, OA cartilage produces a larger amount of NO[61]. Increased levels of nitrate, representing local NO production have been demonstrated in synovial fluid and/or sera from patients with rheumatoid arthritis and OA[62]. The exact role of NO in OA is still uncertain.

The mechanisms involved in the bony responses of OA are not completely understood. Lajeunesse *et al*[63] have hypothesized that abnormal osteoblasts in subchondral bone in OA are a result of increased activity of growth factors and protease's present locally and two systems are involved, the IGF-1 and PA/plasmin systems. As a result of repetitive loading, microfracture and/or the activity of IGF binding protein (IGFBP), there is an abnormal subchondral bone response. This in turn creates cartilage matrix damage in the form of microfractures, repaired by either local synthesis or the release of IGF/IGFBP that stimulates matrix formation in the cartilage. Concurrently, IGF promotes bone cell growth and bone matrix deposition. The IGF-1

system is enhanced in subchondral bone, while PA/plasmin activation promotes local cartilage alteration. Therefore, the local induction in bone and cartilage of IGF-1 and its protease regulatory system promotes both cartilage damage and subchondral bone plate thickening. The imbalance in the repair capacity and damage of cartilage due to subchondral plate thickening subsequently leads to a progressively altered cartilage matrix and eventually to OA. In summary, Lajeunesse *et al*[63] suggest that a bone defect contributes to the onset and/or progression of cartilage degradation in OA.

Moskowitz[64] suggests that subchondral bone responses play a role in OA disease progression, as opposed to disease initiation/development. He suggests that bone can exhibit an early metabolic response but it is the chondrocyte that plays a more important role in OA initiation. Chondrocyte activity in OA cartilage is characterized by increased formation of ECM proteoglycans, collagen and hyaluronic acid which is consistent with a primary role for grow factor related stimulation. Later in the OA process, release of cytokines from synovium and cartilage leads to catabolic cartilage degradation. Increased subchondral bone responses related to growth factors augment the osteoarthritic process with altered biomechanical stresses across the joint. Late stage osteophyte formation they suggest plays a compensatory role in redistribution of forces to provide articular cartilage protection.

1.5.3 Temporomandibular Degenerative Joint Disease

1.5.3.1 Introduction

The vast amount of knowledge of OA in general has created a desire for greater understanding of TMJ degenerative processes. Today, much of our understanding of OA of the TMJ still relies heavily upon research on other synovial joints. However, differences do exist between the TMJ and other synovial joints (e.g. the articular surface

of the TMJ is a dense fibrous connective tissue not hyaline cartilage). The intent of the following sections is to review the OA literature specific to the TMJ.

1.5.3.2 Epidemiology

Degenerative joint disease is found in all populations regardless of ethnic background or geographic location and is present in one to two individuals out of three in any population over the age of 35[65]. DJD in general is considered uncommon in adults under age 40 and becomes extremely prevalent above age 60[66]. Most often patients with TMJ DJD tend to be young females in their second and third decades of life[67-69]. Signs and symptoms of TMJ degenerative disease can also occur in early childhood[70]. The exact prevalence of DJD of the TMJ in society is unknown, but 8% to 12% of patients treated at temporomandibular dysfunction clinics receive a diagnosis of DJD[9].

1.5.3.3 Clinical Features

The signs and symptoms of TMJ DJD include pain, stiffness, reduced range of motion and crepitus[71,72] and can be accompanied by secondary masticatory muscle tenderness. It is not uncommon for DJD to first appear in only one TM joint[73,74], although signs and symptoms may eventually appear in the unaffected joint[71]. Onset of symptoms can be sudden or gradual[71]. A sudden onset is not uncommon as there is a clinically assymptomatic population of DJD patients that given the right circumstances (e.g. trauma) suddenly becomes symptomatic[75]. A more gradual progression of DJD often can present as:

1. Initial pain of the joint after use which is relieved by rest. Morning stiffness of the joint or stiffness after periods of inactivity.
2. More persistent pain often accompanied by muscle splinting or "guarding".
3. Pain on movement or biting.
4. Deviation of the joint to the affected side due to joint restriction.

5. Joint crepitus.
6. Persistent chronic symptoms due to progressive joint breakdown.

Degenerative disease of the TMJ runs a clinical coarse from 1 to 3 years, followed by progressive resolution of symptoms[71,76-78]. Some studies suggest that approximately 70% of patients may experience a remission of symptoms[71,74]. Limited range of movement and crepitus can remain long after subjective symptoms subside[81,82] with one study reporting crepitation being persistent for as long as 6 years[79].

During the early painful stages of DJD there is often restricted joint mobility and mandibular movements, often accompanied by pain in the muscles of mastication and tenderness of the joint capsule[76,80]. Although joint crepitus is a sign of DJD, radiographic evidence of bony change is required to make a definitive diagnosis of DJD.

1.5.3.4 Radiographic Features

Routine radiographic imaging techniques for the TMJ include panoramic, lateral transcranial projections and tomographic imaging, the latter considered the imaging of choice for bony evaluation of the TMJ. During the initial stages of DJD, radiographic changes may not be apparent[74]. Absence of radiographic changes cannot exclude the presence of degenerative change because fibrous articular cartilage is not calcified. In addition, osseous change must be pronounced to be detected radiographically and early degenerative changes in the articular soft tissue may occur long before radiographic signs are visible[81,82].

When early changes are seen radiographically they typically occur on the load bearing area of the condyle[83,84]. Early changes may appear as an inconsistent appearance in the form of the TMJ with a dimuition of superior joint space with flattening of the anterior superior surface of the condyle and an increased radiodensity. Surface erosions may identify active stages with loss of cortication. Small radiolucencies called

"Ely's" cysts may also be noted in the condylar head that may be accompanied by subchondral sclerosis[85]. Advancing stages of the disease are characterized by osteophytes and "lipping" or "beaking" on the anterior aspect of the condyle due to bone deposition[86]. Osteophytes may detach from the articular surface and become "loose bodies" in the synovial space[85]. Very advanced cases of the disease are apparent when the entire functional area of the condyle and eminence looses its regular appearance and the bony contour becomes hazy or distorted. Flattening of the posterior slope of the articular eminence and the condyle including osteophyte formation may be an adaptive response to increase load bearing surface area[87]. Eventually, bony remodeling leads to the end stage of the disease[88].

1.5.3.5 Pathogenesis of TM Degenerative Joint Disease

Even though TMJ DJD has been cited by many as a maladaptation to increased joint loading[89], the pathogenesis of TMJ DJD remains a debatable controversial issue. The intent of the following subsections is to review some of the factors implicated in the pathogenesis.

1.5.3.5.1 Aging

Autopsy studies have suggested that the frequency of TM joint degenerative disease increases in older individuals[90-93]. Age related degenerative changes and decreases in cellular densities of articular cartilage have been reported in mice and rats[94-99]. Age related changes are also seen in the articular tissues of the human condyle[100]. Even though age may be correlated with TMJ DJD, the correlation has not elucidated etiology. Furthermore, in other synovial joints changes observed in articular cartilage from older individuals differ from those observed in articular cartilage from people who have OA[101], and normal life-long joint use has not been shown to cause degeneration[102,103].

1.5.3.5.2 Occlusion

The role of occlusion in temporomandibular disorders (TMD's) has been and remains a controversial issue. Most epidemiological studies have either found no or poor correlation between occlusal interference's and disease in adults[89]. Pullinger *et al*[104] used a multiple regression analysis to compute the odds ratios for 11 different occlusal features as risk factors for the development of five separate TMD's, including osteoarthrosis with disc displacement history and primary osteoarthrosis. They found that although the relative odds for disease were elevated with several occlusal variables, including anterior open bite, overjet greater than 6-7 mm, five or more missing posterior teeth, unilateral maxillary lingual crossbite, and a retruded contact position to intercuspal position slide greater than 2 mm, occlusion was not a dominant factor. They concluded that clinical features such as anterior open bite in osteoarthrosis patients are a consequence of, rather than etiologic factors for, the disorder. Seligman and Pullinger[105] acknowledge that epidemiological studies may possibly demonstrate associations between occlusal factors and DJD but fail to prove the etiologic contributions of occlusion.

1.5.3.5.3 Joint Loading

Unlike synovial joints such as the knee that are loaded in a static state, the articulating tissues of the TMJ are loaded during function[9]. The load bearing areas of the TM joints are the posterior slope of the eminence and the latero-central third of the anterior-superior surface of the condyle. Radiological studies have found that these areas are more frequently associated with DJD[106].

Repetitive microtrauma due to parafunctional habits (eg bruxing or clenching) excessively loads the articular cartilage and, if for extended period of time, may decrease the capacity of the tissues to maintain and regenerate damaged tissue[107]. With the loss of normal tissue repair, tissue breakdown ensues with articular surface

fibrillation, loss of proteoglycans, loss of tissue compressiveness and formation of microadhesions[108,109]. Microtrauma in the presence of disc displacement may exacerbate the repetitive abnormal loading and lead to subchondral bone sclerosis that occurs prior to the onset of TM degenerative disease[110]. It remains unclear whether TM degenerative disease can be attributed to the cumulative effects of repetitive microtrauma.

Major trauma to the joint or mandible may excessively load and damage the articular tissues. Overt trauma has been reported to be linked to degenerative changes of the TMJ[71,111].

Lubrication necessary for normal mechanics of the TM joint comes from three sources: 1) Weeping lubrication, released from the disc and squeezed into the joint space between the loaded bony surfaces and the disc[108]; 2) Boundary lubrication, formed by surfactants such as glycoproteins and phospholipids deposited on the surfaces of the articulating surfaces[112-114] and 3) Synovial fluid produced by the synovial membrane. In a normal TM joint the articular disc is positioned between the osseous articular surfaces during all movements. Its unique position allows for lubrication of the joint surfaces and perfusion of the avascular fibrous connective tissue covering the TM condyle[107]. Altered disc position may affect the perfusion of the articular cartilage, thereby affecting nutrition to this surface[107]. Inadequate joint lubrication may predispose or exacerbate articular tissue failure by increasing frictional resistance during loaded joint movements[9].

1.5.3.5.4 Disc Displacement

The articular disc is a biconcave structure situated between the head of the mandibular condyle and glenoid fossa and composed of dense fibrous connective tissue (collagen Type I). The disc functions as shock absorber and load distributor, aids in

lubrication of joint surfaces by spreading synovial fluid over the articular surfaces, maintains joint stability, and permits rotary motion to occur within the joint[115].

Magnetic resonance imaging (MRI) studies have revealed a ± 30% disc displacement in asymptomatic individuals[116-118], and a ± 86% in individuals that present with joint symptoms[117]. The pathogenesis of disc displacement remains controversial and those factors investigated to determine their role in disc position include macrotrauma, microtrauma, joint hypermobility, occlusal instability, action of the lateral pterygoid muscle and OA[107].

It has been reported that both the condyle and the temporal bone undergo degenerative changes if the articular disc is deranged, perforated or removed[119,120]. Westesson[121] conducted a study with 128 patients that suggests that osteoarthrosis may occur secondary to disc displacement due to the loss of the load distributing ability provided by the disc. This study found osseous changes in 50% of patients with anterior disc displacement without reduction but only minimal osseous change in those with anterior disc displacement with reduction. This author also reported that degenerative changes were consistently found in patients with disc perforation, a finding noted by other authors[122-124]. Anderson and Katzberg[125] have reported similar findings, noting in their study of 141 participants, only 9% of patients with a displaced disc with reduction revealed signs of degeneration, in contrast to 39% of patients with nonreducing disc displacement, and 60% of patients with disc perforation.

Although studies have suggested that DJD occurs secondary to disc displacement and/or disc perforation, others have disputed this. A double blind arthroscopic study of joints with normally positioned discs, displaced but reducing discs and displaced nonreducing discs, perforations were found only in cases with DJD but in 73% the cases with DJD no perforations were found at all[126]. This study implies that DJD may be the cause and perforation is the effect.

Section 1.5.3.5.3 briefly reviewed loading and its potential influence in TM degenerative disease. Degenerative TMJ disease may also occur prior to the onset of disc displacement since changes in the condylar surface due to excessive loading induce degenerative changes in the disc[107]. Stegenga *et al*[127] have proposed that muscle pain and disc displacement are signs of DJD rather than the cause.

1.5.3.6 Articular Cartilage - Structure and Chemistry

A discussion of the pathogenesis of OA needs to include a review of the tissue that is dynamically involved in the OA process. Unique features of the TM condyle articular surface distinguish it form other synovial joints. The purpose of this section is to identify some of these features.

Articular cartilage is an avascular, aneural and alymphatic tissue covering of synovial joints and together with synovial fluid allows for almost frictionless movements and dissipation of mechanical stresses[128]. The articular tissues of the TMJ derive nutrition from synovial fluid, which is stimulated by the alternate loading and unloading of the joint[107]. Articular cartilage of most synovial joints is of the hyaline type, however, the articular surface of the TM joint (mandibular condyle and the temporal fossa) consists of dense fibrous connective tissue (also referred to as fibrocartilage).

The skeletal components of the elbow, hip and knee develop embryologically in a similar manner. They are derived from a solid hyaline cartilage precursor, which form moulds for the future bone[129]. Once the bone is completely formed, hyaline cartilage remains as the articular cartilage[129,130]. Articular cartilage derived in this way, i.e. from the original hyaline precursor, is termed primary cartilage. In contrast rather than being preformed in cartilage, the bones of the TMJ are formed directly from intramembranous centers of ossification[131]. The developing bones become completely surrounded by periosteum, including the future articular surfaces of the TMJ[131]. The periosteum lining these articular surfaces is gradually transformed into the dense fibrous articular tissues of the TMJ[131]. Formed in this way, the articular cartilage of the TMJ is termed secondary cartilage since it is not a remnant of a cartilagenous precursor.

The articular fibrocartilage of the TM condyle is maintained by continual turnover and replacement of cells and matrix produced by chondrocytes within the underlying cartilage[107]. The fibrocartilage of the TMJ consists primarily of Type I collagen[132] rather than the Type II collagen seen in hyaline cartilage[133]. The collagen fibrils make up about 50% of the organic dry weight in hyaline cartilage[133]. In hyaline cartilage approximately 90% of the collagens are Type II, however, Type VI, IX and XI also exist within the ECM[133].

The ECM of both fibrocartilage and hyaline cartilage consists of chondrocytes (as well as fibrocytes in TMJ articular cartilage), collagen, proteoglycans, structural glycoproteins (fibronectin and laminin), water and small amounts of lipid and inorganic components. Water is the major component and constitutes over 65%-70% of the total weight of hyaline cartilage[133] and cited as smaller in TMJ fibrocartilage[134]. Chondrocytes are distributed throughout the ECM accounting for approximately 2%-3% of the total tissue volume in hyaline cartilage[133] and 0.01%-0.1% of the total tissue volume in TMJ articular cartilage[136]. In hyaline cartilage a pericellular and territorial matrix has been

described and surrounds individual chondrocytes, that when in groups are referred to as chondrons[133]. Chondrocytes have the capacity to synthesize as well as degrade all components of the ECM[137].

Beginning at the outermost layer, the articular fibrocartilage of the TMJ can be divided into four layers: 1) The articular zone has collagen fibers organized into sheets and bundles creating a network with a predominantly sagittal orientation[125] with scanning electron microscopy studies also revealing a three dimensional network[121]. This layer has some fibrocytes but no chondrocytes[125]. 2) The proliferative zone, which is mainly a cellular layer of pre-chondroblast cells. 3) The fibrocartilagenous zone, usually the thickest zone, with randomly oriented bundles of collagen fibers[138]. This is a region of cartilage formation that can be further divided into three zones: a) zone of matrix formation, characterized by sparse matrix formation (when compared to hyaline cartilage). Chondrocytes in this zone are progressively displaced from one another as they approach the zone of cellular hypertrophy. b) Zone of cellular hypertrophy where the chondrocytes become expanded, the cytoplasm is vacuolated, but each cell remains enclosed in its separate lacuna. c) Zone of cartilage mineralization and resorption. 4) The calcified cartilage zone. This zone is in direct contact with cortical bone.

Adult human normal hyaline articular cartilage can also be divided into 4 zones or layers[133]. These zones are: 1) The superficial or tangential zone, with chondrocytes that are small, flattened (elongated), oriented parallel to the articular surface and inert with respect to matrix synthesis. This layer has abundant collagen fibers organized horizontally (parallel to the surface) as a mesh: 2) The transitional or middle zone, with large and round chondrocytes: 3) The radial or deep zone, that contain the largest chondrocytes. Both the middle and deeper layers contain vertically oriented (perpendicular to the surface) collagen fibers, separating chondrons (structures that contain vertical aligned ovoid chondrocytes) and their associated matrix. The

chondrocytes in the middle and deep zones exhibit high metabolic activity and are responsible for cartilage homeostasis. 4) The calcified zone. In the deepest layers, the matrix surrounding the chondrocytes shows calcium apatite calcification. The junction between the uncalcified and calcified articular cartilage is commonly called the "tidemark", a thin distinct layer of enhanced calcification that is parallel to the articular surface.

The collagen network gives articular cartilage the tensile strength to maintain tissue shape and volume[139,140] and is enhanced by covalent, intermolecular cross-links formed between collagen molecules[141]. The collagen network also provides a framework for GAG's produced by chondrocytes and counteracts swelling pressure exerted by the hydrophilic proteoglycans within the network[142].

Proteoglycans (at times referred to as ground substance) are the major space filling macromolecules of the ECM of articular cartilage and constitute 15% to 40% of the dry weight of hyaline cartilage[143]. This proteoglycan percentage is thought to be smaller in TMJ articular cartilage[134]. Proteoglycan molecules by their nature are highly hydrophilic and full expansion of the molecules is limited by the inextensibility of the collagen network[142]. Together with the collagen network, proteoglycans allow articular cartilage to undergo reversible deformation[142].

Proteoglycans of synovial joints in general are large macromolecules composed of a central protein core to which GAG's of varying composition and length are covalently attached to the protein core[133]. There are four main groups of GAG's which have been classified: 1) hyaluronic acid; 2) chondroitin sulphate (CS) and dermatan sulphate; 3) heparan sulfate and heparin; and, 4) keratin sulphate. Glycosaminoglycans are unbranched polymers of repeating disaccharide units that are combinations of hexosamines (D-glucosamine or D-galactosamine) and hexuronic acids (D-glucuronic acid, iduronic acid, or L-galactose). They are termed GAG's because one of the two

sugar residues in the repeating disaccharide is always an amino sugar (either N-acetyl-D-glucosamine or N-acetyl-D-galactosamine). In hyaline cartilage, the GAG chains of proteoglycans consist of 90% chondroitin 4 and 6-sulphate and keratin sulphate[133]. In TMJ fibrocartilage, dermatan sulphate content has been cited as being higher and keratan sulphate content is smaller[134].

The major proteoglycan constituent of articular cartilage is aggrecan making up approximately 90% of the mass of proteoglycans[133]. Aggrecan molecules contain three globular domains (G1,G2 and G3), of which the G1 domain can bind covalently to hyaluronic acid, forming large macromolecular aggregates. A specific globular protein known as a link protein[144] stabilizes the interaction between the G1 domain and hyaluronic acid. It is worth noting that hyaline cartilage also contains nonaggregated small proteoglycans such as biglycan, decorin and fibromodulin[145]. They represent only a fraction of the mass of proteoglycans in articular cartilage but may be as numerous as the larger aggregating proteoglycans[133]. Although the exact roles of these smaller proteoglycans is uncertain they are known to interact with collagens, fibronectin and growth factors within the ECM[133].

1.5.3.7 Histology and Biochemistry of Temporomandibular DJD

Unlike other synovial joints of the human body there have been relatively few studies published on the histology and biochemistry of TMJ OA tissues. Of the few published many have incorporated the literature on hyaline lined joints on the basis that the TMJ is also a synovial joint and therefore knowledge of OA in other synovial joints is applicable to the TMJ[134,146]. Fundamental differences exist between the TMJ and other synovial joints (previously reviewed) and while similarities may exist, generalizations may be inappropriate.

Although the exact cause of OA remains unknown, we do know that OA is a multifactorial disease process and does not occur simply when joints are 'worn out'. The degeneration of articular cartilage and changes in the synovial membrane and subchondral bone are central features of OA. The following subsections review the histological and biochemical features of OA specific to the articular cartilage, synovial membrane and subchondral bone of the human TMJ.

1.5.3.7.1 Articular Cartilage

Initial changes in fibrocartilage of the TMJ are characterized by fragmentation of the collagen fibrils resulting in decreased stiffness of the collagen network[147]. The disintegration of the collagen allows for proteoglycan depletion and now combined with increased water content results in localized regions of articular tissue softening (often referred to as chondromalacia)[147,148]. During this period chondrocytes are found arranged in small groups or clusters and electron microscopically show characteristics of increased metabolism, characterized by dilated or vesiculated rough endoplasmic reticulum, well developed Golgi apparatus and secretory vesicles[147]. As the OA process progresses regions of fibrillation (vertical splitting) and irregularities of the articular surface become apparent[149] in addition to horizontal splitting (detachments)[150]. Cluster formation of chondrocytes is often observed adjacent to the splits[150]. In advancing stages pathologic features of chondrocytes include prominent nuclear fibrous lamina, accumulation of intracytoplasmic filaments, swollen mitochondria with distorted cristae and vacuolization of the cytoplasm. Electron microscopically the collagen fibrils show a loose and very disordered arrangement[147]. Ultrastructural pathologic features of the ECM include matrix vesicles and lipid globules derived from necrotic chondrocytes, often containing needle-shaped calcium apatite crystals[147]. Extensive fibrillation of the articular

tissue and eventual denudation of the subchondral bone[151] characterize the advanced stages of OA of the TMJ.

1.5.3.7.2 Synovial Membrane

The synovial membrane of the TMJ is a highly vascularized and aneural layer of connective tissue that lines the surfaces of the joint cavity except the articular surfaces of the condyle, eminence, and the disc[131,152]. The main functions of the synovial membrane are production and removal of synovial fluid and removal of debris from the joint space. The synovial fluid is a transudate from the blood to which is added hyaluronic acid, proteoglycans and proteins produced by the synovial cells[153].

Investigations of synovial fluid of human osteoarthritic TM joints have shown increased levels of GAG components which is thought to reflect evidence of early OA change[154-156]. Light and electron microscopic studies suggest that the initial changes in the synovial membrane of osteoarthritic TM joints include synovial intimal hyperplasia and cell hypertrophy with subsequent deposition of fibrous material in the intimal matrix[68,157]. Increases in the number of active fibroblasts initiates fibrosis of the subintimal layer with fibrosis being the most characteristic feature of OA synovial membranes[68,157]. Although the role of inflammation in the OA process remains unclear, investigations on OA of the TMJ have found that synovial inflammation (synovitis) is almost always present when patients show arthroscopic evidence of DJD[155,158-161].

Since the synovial membrane is not innervated[107] synovitis in itself does not cause pain directly. Pain mediators generated in the inflamed synovial membrane may stimulate capsular nociceptors. As reviewed in previous sections, chemical mediators and cytokines play a major role in OA. Unfortunately, there is little information in regards to the pathophysiological mechanisms that underlie the development of local pain in the TMJ. There have been reports on the influence of neuropeptide Y and serotonin on TMJ

pain and inflammation, but these have been limited to studies on rheumatoid arthritis of the TMJ[162]. Recent investigations have found higher levels of the cytokines IL-1 ra (receptor antagonist) and beta, IL-6, IL-10 and TGF-β1 as well as MMP activity in synovial fluid aspirates of human TM joints with OA compared to normal controls[163-166]. Sandler and colleagues[167] have found a positive correlation between IL-6 concentration and arthroscopically observed synovitis, hypervascularity and redundancy of the synovial tissue in patients with TMJ internal derangement. Elevated levels of nitrate have been reported in the synovial fluid of patients with TMD's[168] and significantly higher levels of NO were demonstrated in patients with internal derangement and OA when compared to controls[169]. These reports appear to indicate that increased levels of NO are involved in the pathogenesis of cartilaginous degeneration of the TMJ.

1.5.3.7.3 Subchondral Bone

The subchondral bone of the TMJ appears to be involved early in the osteoarthritic process. De Bont *et al* [150] in a light microscopic study observed subchondral bone resorption and fibrosis of the adjacent bone marrow that accompanies horizontal splitting of the articular cartilage and clustering of chondrocytes creating degenerative lesions underneath the unaffected superficial articular zone. These early changes are not seen radiographically. Progressive degeneration eventually results in loss of the subchondral cortical layer, bone erosion, and subsequent radiographic evidence of OA[170].

1.5.3.8 Proposed Mechanisms of TMJ DJD

Although the pathogenesis of OA in hyaline joints is not completely understood two general pathways are currently thought to lead OA[60]. The first implies that there is a fundamental defect in cartilage and the matrix of cartilage fails under normal loading of the joint. Therefore OA follows biomechanical failure. The second and most prevalent is

based on the concept that physical forces (repeated microtrauma or single event macrotrauma) cause damage to normal articular cartilage matrix. Two subpathways are involved: a) direct injury of the matrix, and b) injury of chondrocytes embedded in the matrix by the same forces. The chondrocytes in reaction begin to release increased amounts of degradative enzymes and develop inappropriate repair responses.

A maladaptation to increased joint loading has been considered by many researchers to lead to DJD of the TMJ[89]. There have been two concepts proposed for the pathogenesis of DJD. The first is the "TMJ Osteoarthritis Concept"[88,170-171]. Here abnormal joint loading exceeds the functional adaptive capacity of the tissues and changes the equilibrium between form and function. There is subsequent proteoglycan degradation, changes in the synovial membrane, inflammation, and changes in the synovial fluid that lead to impaired lubrication and nutrition of the chondrocytes, ultimately resulting in cartilage degradation. The second is the "Oxidative Stress and Degenerative TMJ Hypothesis"[172,173], a model for the molecular pathogenesis of degenerative TMJ disease. These authors originally advanced three models for TMJ DJD based on the premise that the primary cause of TMJ DJD is abnormal mechanical stress[172]. These models are direct mechanical injury, hypoxia/reperfusion injury and the neurogenic inflammation models. Here excessive mechanical stress could be of magnitude sufficient to damage tissues directly (i.e. direct mechanical injury) or indirectly (i.e. hypoxia-reperfusion injury or neurogenic inflammation). Each mechanism is thought to affect common "downstream" elements involved in tissue loss and pain and include synthesis and activation of proinflammatory cytokines (e.g. IL-1β, IL-6, TNF-α), enhanced arachidonic acid catabolism with the production of PG's and leukotrienes, and synthesis and activation of matrix degrading enzymes (e.g. collagenases, stromelysins, gelatinases). These authors have advanced that abnormal mechanical stresses (e.g. parafunctional habits) lead to the generation of free radicals in articular tissues, creating

an 'oxidative stress'[173]. The free radicals accumulate in the articular tissues and serve as a transduction mechanism for the initiation of molecular events (e.g. proinflammatory cytokine production, protease synthesis and activation, arachidonic acid catabolism), involved in the evolution of degenerative TMJ disease. The mechanical stress may also cause *microbleeding*, leading to hemoglobin deposition in the articular tissues that provides a reactive heme group to catalyze the formation of extremely reactive hydroxyl radicals. The accumulation of these radicals damages or destroys the cartilage matrix with the elaboration of an inflammatory response ultimately affecting the biochemical properties of the articular tissues.

1.5.3.9 Estrogen and DJD

Osteoarthritis in general has a substantially higher prevalence among women than men[66,174]. Osteoarthritis is more common among men than women until around age 50 but becomes much more prevalent among women after 50 and this sex difference in prevalence then increases with age[175,176]. These gender and age related prevalence patterns are consistent with a role of post menopausal hormone deficiency in increasing the risk of OA[174]. Estrogen receptors are found in osteoblasts[177-178] and the effects on prevention of postmenopausal osteoporosis is well established[179-180].

Little information exists about *in vitro* and *in vivo* effects of estrogen on cartilage and its role in the pathogenesis of OA is controversial[181]. Rosner *et al*[182] demonstrated that tamoxifen, an estrogen antagonist, reduces the development of experimentally induced DJD in rabbits. In contrast, estradiol facilitates the process. A study using animals has suggested that the upregulation of estrogen receptors in cartilage might initiate the osteoarthritic changes[183]. The authors of this study found both the chondrocyte number and proteoglycan concentration were significantly reduced in the superficial layer of cartilage in estradiol-injected knee joints. Estrogen has been found to

inhibit cartilage in animal models of OA[184] and increase the production of proinflammatory cytokines in uterine tissue[185] and by macrophages[186]. Some believe that the importance of estrogen in OA may not be mediated through cartilage but rather through bone where it is known to prevent the activation of osteoclasts[187].

Temporomandibular disorders in general have there highest prevalence among women in their reproductive years[188] and 80% of treated cases of TMD are women[188,189]. Between 8% to 12% of patients that present to a TMD and Orofacial pain clinic receive a diagnosis of TMJ degenerative disease[9]. It is well recognized that these patients tend to be young females in their second and third decades of life[67-69,88]. The influence of endogenous reproductive hormones may play a role as the age of onset of TMD conditions is generally after puberty, prevalence rates are higher in women than men, and prevalence is lower for women in the post menopausal years than those of reproductive age[190,191]. Recently a study comparing post-menopausal hormone use among 1291 women over age 40 to 5164 controls found that the odds of being a TMD patient were approximately 30% higher among those receiving estrogen compared to those not exposed[191]. Little is known in regards to the influence of estrogen in TMJ DJD, however, estrogen receptors have been identified in the TMJ of female baboons[192] and rats[193] but not male baboons[194]. Ng *et al*[193] in their study of resected TM condyles from female rats cultured for 4 days in media with different estradiol concentrations reported a qualitative decrease in chondroblast, thickness of condylar fibrocartilage, and significant decrease in proteoglycan content. Further research is required to clarify the role of estrogen in TMJ DJD.

1.5.3.10 Overview of Treatment of TMJ DJD

Conservative treatment may involve reducing joint loading by advising patients to avoid parafunctional habits (clenching, bruxing and gum chewing) and modifications in

diet (soft foods, small bites). Physiotherapy may also be advised in which modalities such as ultrasound and transcutaneous nerve stimulation (TENS) may used to control inflammation, decrease secondary muscle spasm and improve joint mobility. The physiotherapist may also suggest various home exercises.

Pharmacological approaches are an important conservative adjunct for treating the symptoms of DJD when pain is an issue. Nonsteroidal anti-inflammatories such as ibuprofen, have traditionally been the medicines of first choice[195,196]. Muscle relaxants may also be prescribed in those situations were myalgia may be a factor secondary to the DJD.

Occlusal splint appliances are often advocated for patients with TMD. Splint therapy may prove palliative for those patients when secondary masticatory myalgia is associated with DJD.

Injections into the TMJ may be a treatment modality for patients with symptoms refractory to more conservative approaches. Intraarticular (i.a.) injection of corticosteroids have been shown to provide symptom relief [197,198] but multiple injections should be avoided since they have been associated with tissue necrosis[199]. Hyaluronic acid functions as a lubricant in normal synovial fluid. Intracapsular injection of sodium hyaluronate has been shown to provide pain relief in patients with non-reducing disc displacements[200,201] but are no better than placebo injections in patients with DJD[202].

Surgical intervention is rarely indicated for degenerative diseases of the TMJ because the disease process is self-limiting in a high percentage of cases[203]. Minor surgical interventions such as arthrocentesis may be suggested only if conservative efforts to control pain fail[204].

1.5.4 Nonsteroidal Antiinflammatories

1.5.4.1 Historical Overview

The bark of the willow tree has been known for centuries to have medicinal effects with its ability to relieve fever noted by Reverend Edmund Stone of England in 1763[205]. Approximately 50 years later, salicin, a glycoside of salicylic acid and the first known NSAID and precursor to aspirin, was isolated from willow bark[205]. Subsequent synthesis and mass production of sodium salicylate was used for treatment of rheumatic fever[205].

Acetylacetic acid (ASA) was discovered in 1898 by a chemist at the Bayer division of I.G. Farber[205]. At 4 grams per day it was found to relieve fever and pain and reduce inflammation[205]. Acetylacetic acid has the distinction of being the most used drug this century, billions of tablets consumed daily, and still considered the standard against which all NSAID's are compared to determine efficacy[206]. It is estimated that approximately 2% of North Americans use NSAID's on a daily basis[207] and 60 to 100 million prescriptions are written per year in the United States alone[208-209]. The term "nonsteroidal anti-inflammatory" drug was first applied to phenylbutazone in 1949[210].

1.5.4.2 Classification

There are a very large number of NSAID's manufactured and marketed in North America. It would not be meaningful to the reader to divide them into their chemical classifications and list all their names. It is more important that the reader is aware that approximately 18 different NSAID's are prescribed in Canada that fall into one of nine main categories presented in Table 1.1[211].

TABLE 1.1

NSAID CLASSIFICATION

CHEMICAL CLASS	DRUGS	TRADE NAME
Salicylic acids	Acetylsalicylic acid	Aspirin
	Diflunisal Choline	Dolobid
Propionic acids	Ibuprofen	Motrin
	Naproxen	Naprosyn
	Flurbiprofen	Ansaid
	Fenoprofen	Nalfon
	Tiaprofenic acid	Surgam
Phenylacetates	Diclofenac	Voltaren
Pyranocarboxylic acid	Etodolac	Ultradol
Pyrazolones	Phenylbutazone	Butazolidin
Indoleacetic acids	Indomethacin	Indocid
	Sulindac	Clinoril
	Tolmetin	Tolectin
Oxicams	Piroxicam	Feldene
	Tenoxicam	Mobiflex
Alkanones	Nabumetone	Relafen
COX-2 selective	Celecoxib	Celebrex
	Rofecoxib	Vioxx

1.5.4.3 Pharmacokinetics

Nonsteroidal antiinflammatories chemically are weak acids or metabolized to weak acids[212]. Even though chemical similarity between NSAID's lead to common pharmacokinetic properties (absorption, distribution and elimination), there are still considerable differences in some pharmacokinetic and to some extent pharmacodynamic profiles[212].

Most NSAID's are almost completely absorbed from the GI tract, their hepatic clearance is low and as a consequence there is little first pass metabolism by the liver. As a result, most NSAID's have a high bioavailability after their oral administration[212]. Aspirin and diclofenac are an exception to this since they are subject to considerable first pass metabolism[207,212]. Peak serum concentrations (Cmax) generally occur within 2 to 3 hours post-administration. Absorption is quicker when taken on an empty stomach, but potential gastric irritation makes it necessary to take them with meals[212].

Nonsteroidal anti-inflammatory drugs like many acidic drugs are highly protein bound (>90%) but most strongly bound to albumin in plasma, and their volumes of distribution low[207]. Their acidic nature, fortunately, makes them amenable for effective distribution into cells with acidic environments like those of inflamed joints through the process of ion-trapping[212]. The concentration of NSAID's in these tissues is thought to be responsible for some of the characteristic therapeutic and adverse effects of NSAID's[213].

Peak serum concentration of NSAID's are generally achieved within 2 to 3 hours post-administration (Table 1.2). Based on their half-life, NSAID's can be divided into 3 categories: those with half-lives less than 6 hours, those with half-lives of 6-12 hours and those with half-lives in excess of 12 hours[212]. Nonsteroidal anti-inflammatory drugs with long half-lives are usually administered once or twice a day with minimal plasma concentrations fluctuating resulting in a relatively constant pharmacological effect[212].

TABLE 1.2

PHARMACOKINETICS OF NSAID'S

DRUG	ABSORPTION PEAK PLASMA LEVEL (hours)	HALF-LIFE (hours)	EXCRETION
Acetylsalicylic acid	1-2	0.25	Renal
Diflunisal choline	2-3	7-8	90% renal
Ibuprofen	1-2	2	50%-75% renal
Naproxen	2-4	12-15	95% renal
Flurbiprofen	1.5	3-9	Renal
Fenoprofen	2	3	Renal
Tiaprofenic acid	0.5-1.5 hours	1.7	Renal
Etolac	0.8-2.0	3-11	72% renal, 16% fecal
Phenylbutazone	2.5	84	Renal
Indomethacin	2	4-11	60% renal, 30% fecal
Sulindac	2	16	50% renal, 25% fecal
Tolmetin	0.5	1-3	Renal
Piroxicam	2-5	38	67% renal, 33% fecal
Tenoxicam	1.25	72	60% renal, 17% bile
Nabumetone	2.5-4	23-30	Renal
Diclofenac	2	1.8	60% renal, 40% bile and fecal
Celecoxib	3	11	57% fecal, 27% urine
Rofecoxib	3	17	72% renal, 14% fecal

These drugs also tend to accumulate in the body for a prolonged period (e.g. Tenoxicam has a half-life of about 72hours) and changes in the rate of absorption tend to have little effect on their plasma concentrations during long-term therapy[212]. Nonsteroidal anti-inflammatory drugs with shorter half-lives including ibuprofen and indomethacin and administered every 6-8 hours. Plasma concentrations of these NSAID's fluctuate over a dosage interval because the dosage intervals are longer than their half-lives, however, analgesic and anti-inflammatory activity is found to be well maintained[212]. Many short half-life NSAID's are available in a sustained release formulation so that the absorption profile and consequently the clinical effect of the drug are prolonged[207].

It is thought that a major site of action of NSAID's is in the synovium[212]. They are slowly transferred between plasma and synovial fluid with concentration between the two sites influenced by their individual half-lives of elimination[207]. Total mean concentration in synovial fluid is approximately 60% of the mean drug concentration in plasma. This difference is due to the lower levels of albumin in the synovial fluid that results in reduced binding of NSAID's[207].

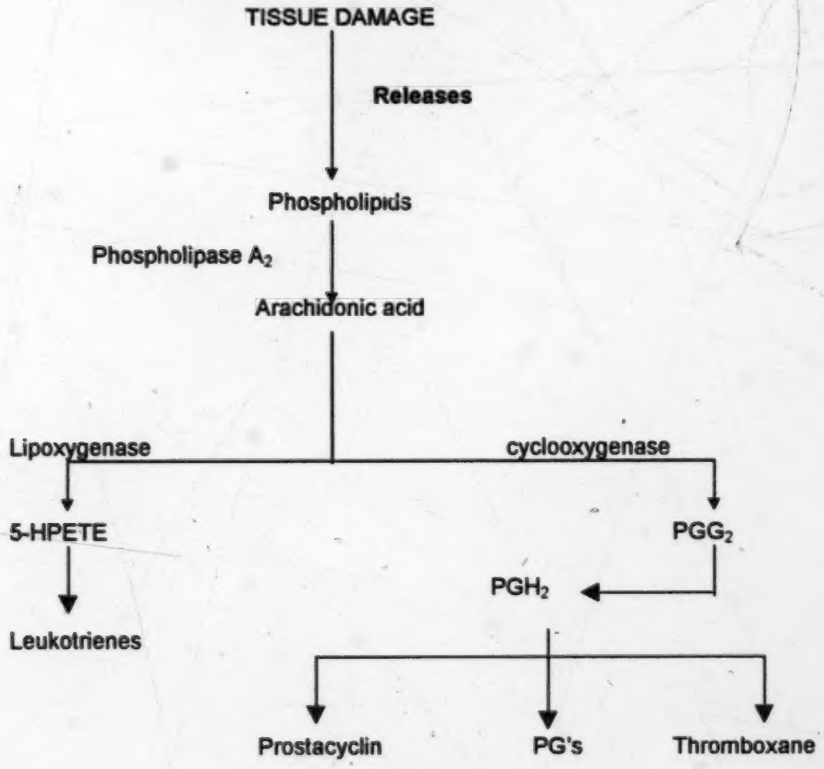
Nonsteroidal anti-inflammatory drugs are cleared predominantly as inactive metabolites by hepatic metabolism either through oxidation or glucuronide conjugation[207,212]. Only small proportions of NSAID's are excreted unchanged in the urine[207].

1.5.4.4 Pharmacodynamics

The first hypothesis to explain the effects of NSAID's was proposed by John Vane[214]. He demonstrated that NSAID's inhibited the enzymatic synthesis of prostaglandin's (PG). Today it is generally accepted that NSAID's work specifically by inhibition of the enzyme PG endoperoxidase synthase (PGHS) or cyclooxygenase (COX)[214]. Cyclooxygenase is reported to have two catalytic sites (Figure 1.1)[214]. The

FIGURE 1.1

THE CYCLOOXYGENASE AND LIPOXYGENASE PATHWAYS OF ARACHIDONIC ACID CATABOLISM



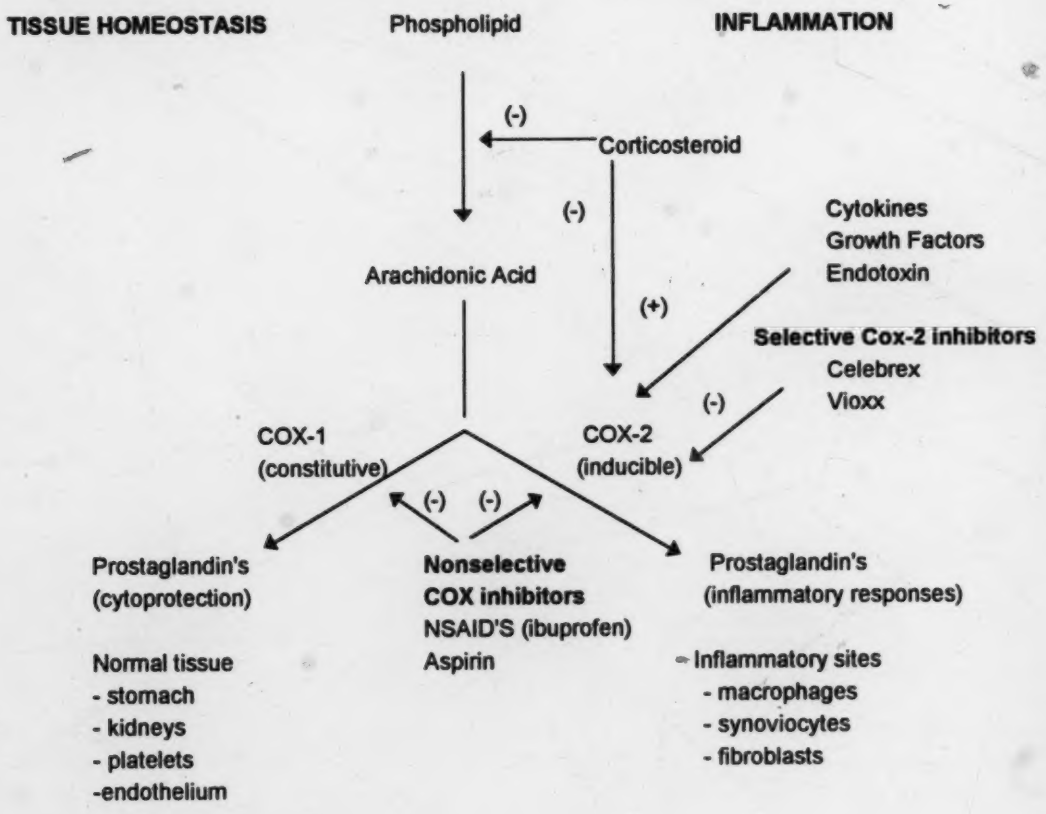
HPETE = hydroperoxyeicosatetraenoic acids; PG = prostaglandin

first, described as the COX active site, converts arachidonic acid to the endoperoxide, PGG_2. The second, described as the peroxidase active site, then converts the PGG_2 to another endoperoxide, PGH_2. PGH_2 is then further processed by specific isomerases forming the PG's, prostacyclin, and thromboxane A2. Inhibition caused by aspirin is the result of the irreversible acetylation of the COX site of PGHS, leaving the peroxidase activity of the enzyme unaffected.

In the early 1990s, researchers discovered two isoforms of the COX enzyme: COX-1 and COX-2[215] (Figure 1.2). The genes for the two forms are on separate chromosomes. They are present in various amounts in different organs, and serve distinct biological functions[216]. Cyclooxygenase-1(or PG H synthase-1) has been described as the "housekeeping" enzyme regulating normal cellular processes and is stimulated by hormones or growth factors[217]. Cyclooxygenase-1 is constitutively expressed by normal cells[218] and PG's synthesized by this enzyme are thought to be important in maintaining physiological function of tissues[218]. For example, COX-1 activity is thought to be important in maintaining the integrity of the gastric and duodenal mucosa from damage induced by toxins such as NSAID's and alcohol[218,219]. Cyclooxygenase-1 is inhibited by virtually all known NSAID's (with exception of the newer COX-2 specific NSAID's) to varying degrees and this inhibition is considered the primary reason for the toxic effects of NSAID's on the GI and renal systems[214,217,219]. Cyclooxygenase-2 (or PG H synthase-2) is an inducible enzyme of inflammatory cells[218] that is usually undetectable[214,217]. Inflammatory stimuli and cytokines increase its expression. The anti-inflammatory actions of NSAID's are caused by the inhibition of COX-2.

FIGURE 1.2

THE ROLE OF CYCLOOXYGENASE ISOFORMS 1 AND 2 IN NORMAL AND INFLAMED TISSUES[from 251]



1.5.4.5 Central Nervous System Effects

It is well established that NSAID's have antipyretic, analgesic and anti-inflammatory properties. NSAID's are generally considered peripherally acting analgesics but also have effects in the spinal cord and brain. This influence of NSAID's on the central nervous system (CNS) has been reported by Catania *et al*[220]. They found that when lysine acetylsalicylic acid and sodium salicylate were injected into the lateral cerebral ventricle, they inhibited edema induced in the mouse ear by topical application of picryl chloride. Systemic injection of doses that were effective centrally did not affect inflammation indicating that the inhibitory activity they observed was not due to systemic escape of the drug. On the other hand, central administration of indomethacin that is anti-inflammatory when given intraperitoneally did not inhibit peripheral inflammation suggesting that it lacks the central anti-inflammatory effect of salicylates. This observation, in addition to a lack of an anti-inflammatory effect with a PG inhibitor (dexamethasone) or pro-inflammatory influence with PGE_2, suggested that not all the effects of NSAID's can be explained by the inhibition of PG synthesis and PG's may not be important to central modulation of inflammation. Several lines of evidence, however, implicate the inhibition of central PG synthesis in analgesia, particularly in models with a strong hyperalgesic component to their pathophysiology[221]. The mechanisms involved in analgesia may be a COX independent inhibition of central hyperalgesia induced by glutamate and substance P[221].

Taiwo *et al*[222] reported that intrathecal injections of PGE_2 in rats antagonized the analgesia produced by both brain stimulation and intracerebroventricular morphine. In contrast NSAID's (indomethacin and acetylsalicylic acid) synergized with brain stimulation and morphine-induced analgesia. In addition, phentolamine (alpha-adrenergic antagonist) and 6-hydroxydopamine (catecholaminergic selective

neurotoxin), that block tonic catecholamine activity in endogenous opioid-mediated analgesia systems, prevented the hyperalgesia induced by intrathecal PGE_2. These results demonstrate PG's ability to inhibit the pain suppressing influences of spinal noradrenergic synapses. NSAID's with central effects may be reducing pain by eliminating these effects of PG's. The exact mechanisms involved remain to be clarified.

There have been other reports for a CNS involvement with various NSAID's[221,223-225]. Much of the data to support a central analgesic activity of NSAID's has come from animal pain models[224,226,227]. Using the nociceptive flexion reflex model[228] various NSAID's (i.e. indomethacin, ibuprofen, ketoprofen) have demonstrated a CNS analgesic activity in humans[229-231].

1.5.4.6 NSAID's and Osteoarthritis

Despite nonpharmacological treatment strategies (reduced joint loading, exercise, physiotherapy, surgery, mobility aids, etc.), pharmacological therapy remains a commonly employed method of symptom relief for OA[232]. For decades, NSAID's have provided relief of joint pain and improved mobility for millions[233] with scientific evidence supporting the efficacy and superiority of numerous different NSAID's to placebo for symptomatic treatment for OA[234]. The use of NSAID's continues to grow[217], with 60 to 100 million prescriptions annually in the U.S.[208-209]. Research papers continue to be published reporting new information on their biologic effects[217], pharmacokinetic and pharmacodynamic profiles[235] and mechanisms of action[214]. Over the last ten years, however, there has been increasing evidence that NSAID's may be hazardous, at times making their use controversial. It has been suggested that the availability of such a large number of NSAID's may be evidence of the failure of any to diminish major side effects[236].

1.5.4.6.1 Side Effects

This section is not intended to argue whether these hazards outweigh the therapeutic benefits of NSAID's, but to enlighten the reader to some of the important issues that have arisen from their use

Many people taking nonselective COX NSAID's experience mild side effects such as dyspepsia, heartburn and generalized abdominal discomfort[236]. Major side effects associated with NSAID's include renal insufficiency, GI bleeding or ulceration, congestive heart failure, hypertension and hyperkalemia[237-241] with the elderly being at greatest risk for developing these adverse effects. Gastrointestinal upset and ulceration is considered the most frequently reported side effect for patients with OA taking NSAID's[234]. NSAID therapy has been associated with a GI ulceration prevalence of 12% to 30% for the stomach and 2 to 19% for the duodenum[236]. They have been implicated in causing up to one-third of all serious peptic ulcers[242]. The elderly are at greatest risk to develop OA, and because the prevalence of side effects is age related, the elderly are also at greatest risk of developing GI symptoms, ulceration, hemorrhage and death as a result of inhibition of the synthesis of protective PG's[243,244]. The risk of complications in the elderly increases with higher doses and is highest in the first month of usage[245]. It has been reported that among the elderly, the annual rate of hospitalization for ulcers as a result of NSAID use is 16/1000, 4 times greater than non NSAID users[239]. In addition, 30% of all hospitalizations and deaths of people over the age of 65 is due to NSAID use[239,245,246]. Epidemiological studies support the fact that chronic use of NSAID's results in a high incidence of GI ulceration's, which are associated with major and serious complications such as perforation, bleeding and death[243,247]. In the United States alone, there are an estimated 41,000 hospitalizations and 3,300 deaths each year among the elderly that are attributable to NSAID's[248].

1.5.4.6.2 Cyclooxygenase1 and 2

Today there is much interest in COX-2 selective NSAID's for treatment of arthritis. Theoretically COX-2 selective NSAID's should decrease inflammation but not influence normal physiologic functions and thus cause fewer GI side effects. Two COX-2 selective inhibitors, MK-966 (Vioxx®, Merck and Co.) and celocoxib (Celebrex®, G.D. Searle and Co.), have been studied in Phase II and III clinical trials in OA, rheumatoid arthritis, and dental pain models[249]. In a 2-week OA trial, 293 patients with painful OA of the knee were randomized to placebo or celecoxib[250]. Patient and physician global assessments, an OA severity index, patient assessment of arthritis pain using a visual analog scale and a functional-capacity scale assessed symptoms. Celecoxib therapy was associated with greater symptomatic improvement when compared with placebo. Patients in the celecoxib treatment group also experienced a significant benefit in their health-related quality of life[Zhao et al in 251]. Adverse events observed in patients who received celecoxib were similar to those in the placebo group. In a 6-week, multicenter, placebo-controlled trial involving 672 patients with knee and hip OA, MK-966 outperformed placebo as measured by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain scale[Ehrich in 249]. No significant difference between treatment and placebo groups was seen with respect to the incidence of patient discontinuation because of adverse effects.

1.5.4.6.3 Effects on Articular Cartilage

For a number of years there have reports in the literature that NSAID's worsen OA because of their effects on cartilage metabolism via inhibition of proteoglycan biosynthesis. However, there is no general consensus that all NSAID's cause cartilage damage. Numerous *in vitro* studies have shown that some NSAID's, for example acetylsalicylic acid, fenoprofen, ibuprofen, sodium tolmetin[252-254] inhibit the net synthesis of proteoglycans by chondrocytes *in vitro*, while others such as diclofenac,

indomethacin, naproxen[255], tiaprofenic acid and piroxicam[256] have no *in vitro* effect. On the other hand sulindac sulfide has shown to have a slight stimulatory effect and benoxaprofen markedly stimulated proteoglycan synthesis *in vitro*[257]. These results are not in total agreement with Dingle[258] who reported that in an *in vitro* assessment of NSAID action on 300 patients' femoral head cartilage synthesis. This author reports that nimenezulide, ibuprofen, indomethacin and naproxen depress GAG synthesis in a statistically significant way, diclofenac, piroxicam, aspirin and nabumetone have no major effect and acelofenac, tenidap and tolmetin were stimulatory.

In vivo animal models using dogs that were fed aspirin have found that cartilage degeneration is much more marked than those dogs not fed aspirin[259]. In addition, the proteoglycan concentration of the cartilage matrix was significantly reduced in those dogs fed aspirin and the augmentation of proteoglycan synthesis in the OA cartilage, reflecting repair activity was virtually eliminated. Oral administration of aspirin has also been found to accelerate OA in black mice genetically predisposed to this disease[17].

Recently, Huskisson *et al.*[18] reported the results of the effects of the NSAID's, indomethacin and tiaprofenic acid versus placebo on 812 patients with OA of the knee. Their study found patients taking indomethacin at 25 mg three times a day for at least a year were 2 times more likely to have an increased rate of radiological deterioration of joint space than those patients taking placebo only. On the other hand no difference in rate of deterioration was seen with tiaprofenic acid when compared to placebo.

The use of NSAID's and its role in advancing OA remains controversial. It's apparent, however, that long-term use of non-selective NSAID's can result in serious side effects (notably GI) and evidence is mounting that some NSAID's may effect the progression of OA at the level of the chondrocyte. These issues have created research for newer medications, those that have reduced toxicity profiles and cartilage sparing. Newer NSAID's as reviewed previously, are found to have improved side-effect profiles

over current NSAID's due to their selective inhibition of the COX-2 enzyme. Food supplements such as GS and CS are also being investigated and discussed in the following sections.

1.5.5 Glucosamine Sulphate

1.5.5.1 Physical Properties and Pharmacokinetics:

Glucosamine (2-amino-2-deoxy-alpha-D-glucose) is a naturally occurring aminomonosaccaride of almost all human tissues, including cartilage. It is synthesized in the body by addition of an amino group to glucose and used to form glycoproteins and GAG's[20]. D-glucosamine is the hexosamine component of GAG's. Hyaluronic acid, keratin sulphate and heparan sulphate are composed in part of repeating units of glucosamine[260].

Glucosamine is a small molecule with a molecular weight of 179.17[261]. Commercial preparations are in the form of the sulphate salt, GS. After oral, intramuscular (i.m.) or intravenous (i.v.) administration GS is rapidly absorbed and split into D-glucosamine (DG) and sulphate ion[261]. At 37°C (human body temperature), DG has a pka of 6.91, and with the human intestinal pH 6.8, 54% of DG is non-ionized. At pH 7.4 (human blood) 75% is non-ionized. The pka of DG therefore makes it favorable for absorption across the lumen of the intestine and then diffusion amongst the cells of the body[262].

The pharmacokinetics of glucosamine have been investigated by Setnikar[261,262,26] using ^{14}C labeled glucosamine administered to dogs, rats and humans either by i.v., i.m. or oral route. Following oral administration of GS, close to 90% is absorbed in the human intestine and free glucosamine is not detectable in plasma[262]. Maximum urinary excretion of radioactivity after oral administration is achieved between 4 and 8 hours, with a $t_{1/2}$ of approximately 3 hours. Eight hours after administration 77% of the total excreted radioactivity is recovered. Fecal excretion of radioactivity accounts for 11% of the administered oral dose, and occurs mainly between 24 and 72 hours after administration. One hundred and twenty hours following oral administration 11% appears in the feces and 10% appears in the urine[261]. The radioactivity excreted with the feces after oral administration is thought to represent the non-absorbed fraction of DG, and therefore after oral administration GI absorption is almost 90% of the administered dose. Studies with dogs have found that DG rapidly diffuses into most tissues and organs with a special tropism for articular tissue of the femoral head[261].

1.5.5.2 Pharmacodynamics

An *in vitro* study in the 1950's showed that glucosamine stimulated the uptake of $^{35}SO_4^-$, a marker of GAG synthesis by cartilage[263]. Glucosamine sulphate has been found to significantly increase *in vitro* secretion of GAG's by fibroblast cultures[264]. Other research in the 1970's and early 1980's have also demonstrated that exogenous glucosamine increased the synthesis of GAG's in cartilage cultures[265,266]. More recently, using chondrocytes isolated from and cultured from human osteoarthritic femoral heads has found that GS induces a significant and dose dependent increase of proteoglycan synthesis but did not effect deoxyribonucleic acid (DNA) synthesis or collagen Type II or PGE_2 production by chondrocytes[267].

Using animal models of inflammation oral glucosamine was reported to protect rats from inflammation caused by several non-specific foreign agents (dextran, formalin and acetic acid) but does not exert activity against specific mediators of inflammation (histamine, serotonin or bradykinin)[268,269]. Glucosamine sulphate has no analgesic activity, is ineffective against proteolytic enzymes of inflamed tissues and against the biosynthesis of PG's elicited from arachidonic acid or histamine[268,269]. Glucosamine sulphate reduces superoxide radicals generated by macrophages, inhibits lysosomal enzymes and its effects are PG independent[268,269]. Glucosamines effects have been described as a PG independent, COX independent "antireactive" activity[268,269].

1.5.5.3 Glucosamine Sulphate for Osteoarthritis

Over the past five years public interest in GS for treatment of OA has increased due in part by two non scientific publications, *The Arthritis Cure*[270] and *Maximizing the Arthritis Cure*[271]. The lack of interest in GS by researchers and pharmaceutical companies in general has been attributed by some to the fact that glucosamine is a natural product that cannot be patented[272].

The potential of glucosamine as a therapeutic agent for OA was first reported in 1969 by a German physician[273]. Approximately a decade later other German investigators[274-276] reported decreases in pain often accompanied by increased mobility when patients received a 400 mg solution of GS once daily administered either i.v., i.m. or i.a. These results should not be considered definitive since they were uncontrolled studies. Numerous controlled, double-blind investigations evaluating glucosamine (oral, i.v. or i.m. administration) versus placebo in patients diagnosed with OA of the knee were carried out in the early 1980's[277-280]. All studies reported gradual and progressive reduction of articular pain, joint tenderness and swelling, and improvement in the range of motion. A double-blind eight week study involving 40 patients with OA of the knee,

found that GS 500 mg tid was as effective as ibuprofen 400 mg tid in relieving pain after the first two weeks and by the end of the trial was more effective[281].

Many of these earlier studies reported improvement in symptoms when patients with OA of the knee were administered glucosamine, but there were limitations in study design that included using hospitalized patients undergoing active physiotherapy, blinding placebo injections and short study times. These earlier studies have been critically reviewed[282-284].

A number of articles on GS have been published within the last decade. Muller-Fassbender and colleagues [285] in a double blind 4-week trial, randomized 200 patients with OA of the knee. These researchers found oral GS (500 mg tid) just as effective as ibuprofen (400 mg tid) from the second week of treatment and no difference was found between groups with respect to the magnitude of response. Adverse events were found to occur in 35% of the ibuprofen group, but in only 6% of the GS group, with fewer drop-outs in the latter. Qiu *et al*[286] in a similar 4 week trial of 178 Chinese patients found both GS (1500mg daily dose) and ibuprofen (1200mg daily dose) significantly reduced the symptoms of knee OA with a trend toward GS to be more effective. The GS group reported fewer adverse reactions (6%) compared to ibuprofen (16%) group and there were no drop-outs in the GS group as compared to 10% in the ibuprofen group. Noack and colleagues[287] in a 4 week placebo controlled study of 252 patients with OA of the knee reported patients that had taken 1500 mg/day of GS orally showed significant improvement in the Lequesne index compared to the placebo group. Reichelt *et al*[288] compared treatment of 400 mg of GS i.m. twice per week for 6 weeks with placebo injections administered on the same schedule in 155 patients with OA of the knee. Fifty-five percent of patients who received GS and 33% of those patients given placebo responded as judged by the Lequesne index.

In the most recent study published, patients with OA of the knee in an 8 week double blind study were given either glucosamine hydrochloride (n=41), 500mg three times per day, or placebo group (n=48)[289]. No statistically significant difference was found between groups in the primary endpoint measured (WOMAC pain score) between Week O and Week 8. However, significant differences from Week 5 to Week 8 in secondary endpoints (daily diary and knee examination) were found that suggested that glucosamine hydrochloride benefits some patients with knee OA.

There has been only one published report of GS for patients with TMJ OA[290]. The primary outcome of this study of 50 patients was a reduction in joint noise. All patients received glucosamine hydrochloride (1600 mg twice daily), 1000 mg of calcium ascorbate (1000 mg twice daily) and a mixture of CS-4 and CS-6 (1200 mg twice daily). Eighty percent of patients after an undefined period of time reported a reduction in joint noise. Unfortunately, this study does not indicate treatment time (other than patients were re-evaluated every 2-3 weeks) and cannot distinguish which of the co-administered supplements influenced outcome the most. In addition, the influence of either occlusal splint therapy introduced during the study for "many" patients, or ibuprofen and aspirin permitted (counts not reported) when joint pain and/or swelling interfered with daily routines and activities, on the primary outcome is unclear. This study was neither randomized nor blinded. Reducing joint noise as a primary treatment outcome for TMD patients is questionable and the value of this study is uncertain.

1.5.6 Chondroitin Sulphate

Chondroitin sulphate is a GAG composed of the monosaccharides N-acetyl-D-galactosamine and D-glucuronic acid with sulphate groups located at either C_4 or C_6[291]. There are approximately 60 repeating units per molecule of CS. It is present predominantly in the ECM surrounding cells and is most abundant in those tissues with

large ECM such as those that form the connective tissues of the body, cartilage, skin, blood vessels and also bone, ligaments and tendons.

The mechanism of action of CS may be similar in nature to GS, since it can also provide substrates for proteoglycan synthesis. Using cultured articular chondrocytes from humans Bassleer *et al*[280] have found that CS induces a significant and dose dependent increase of proteoglycan synthesis and significantly increased amounts of proteoglycan in chondrocyte clusters. Chondroitin sulphate does not, however, effect collagen Type II or PGE_2 production or DNA synthesis by chondrocytes. Cell culture studies have shown that exogenously supplied CS competitively inhibits the action of metalloproteases in the cartilage matrix[Ronca in 292], decreasing the degradation of collagen and proteoglycans.

Chondroitin sulphate has been investigated in the treatment of arthritis, typically using proprietary CS products. The most commonly investigated products are referred to in the literature as GAG polysulphate (Arteparon), galactosaminoglycuronogylcan sulphate (Matrix), CS (Condrosulf), and CS(Structum). Arteparon has been used in veterinary medicine in Europe for over two decades for the treatment of DJD[293]. The drug is administered directly into the diseased joints to improve functional properties of the cartilage and to stimulate cartilage metabolism. Arteparon administered i.a. or i.m. in humans with OA of the hips resulted in reduction of pain, and improved function and mobility[294]. Matrix given orally (800 mg/day) for OA of the hands[295] and i.m. for tibiofibular arthritis of the knee[296] produced improvement in arthritic symptoms regardless of the manner of administration.

Oral administration of CS (Condrosulf), 800 mg or 1200 mg per day, proved more effective than placebo for patients with OA of the knee[297-299] and finger joint[300]. Morreale *et al*[301] conducted a randomized, multicenter, double-blind clinical trial to assess the efficacy of CS administered orally in comparison with the NSAID diclofenac sodium. The

patients treated with diclofenac showed prompt reduction of clinical symptoms, however, symptoms reappeared quickly after the discontinuation of treatment. Patients treated with CS had a slower response to treatment, although the favorable response remained up to three months after discontinuation of treatment.

Chondroitin sulphate is a large molecule with a molecular weight is about 50000 and oral absorption has been questioned by some researchers[302]. Whereas more than 90% of GS is absorbed, it is thought that the absorption rate of CS is between 0% and 8% after oral administration[Morrison(1977) in 293]. The pharmacokinetic properties of CS (Condrosulf) were investigated by Conte *et al*[303]. Significant extraction procedures were utilized to generate a low molecular mass product, which could be characterized for structure, physiochemical properties and purity. Only the fraction with a relative molecular mass of 14250 Daltons with a purity of 97% was used in their experiments. The preparation was radioactively labeled and administered by oral route in the rat and dog. Although more than 70% of the radioactivity was absorbed and was subsequently found in urine and tissues, the radioactivity associated with the intact molecule of CS corresponding to the molecular mass of the administered dose was relatively small (app. 8.5%) and decreased rapidly over time. The majority of the radioactivity absorbed was actually associated with molecules with a molecular mass of less than or equal size to N-acetylgalactosamine (one of the two constituent monosaccharides comprising the polysaccharide chain of CS). This radioactivity increased over time and remained elevated. Radioactivity after 24 hours was highest in the small intestine, liver and kidneys, with relatively high amounts found in joint cartilage, synovial fluid and trachea.

The intact absorption of CS subsequent to an oral dose is a controversial subject[293]. It's generally considered a physiological fact that molecules with a high molecular mass and charge density cannot pass through gastric and intestinal mucosa intact[293]. Available data seem to partially refute this belief since some findings indicate

as much as 8.5% of an oral dose can be absorbed intact under some circumstances[293]. However, the majority of the physiological benefits subsequent to administration of CS appear to be a direct result of increased availability of the monosaccharide building blocks (glucuronic acid and N-acetylgalactosamine) created by the hydrolysis of CS into smaller molecules during digestion and absorption[293].

1.6 References

1. Kelsey JL , Hochberg MC. Epidemiology of chronic musculoskeletal disorders. Ann Rev Public Health 1988; 9: 379-401.
2. Scott JC, Hochberg MC. Arthritis and other musculoskeletal diseases. In Brownson RC, Remington PL, Davis JR editors. Chronic disease epidemiology and control. Washington DC: American public health association; 1993: 285-305.
3. Keuttner KE, Goldberg VM, editors. Osteoarthritic disorders. American Academy of orthopedic surgeons, Rosemont;1995: xxi-v
4. Brandt KD, Mankin HJ, Shulman LE. Workshop on etiopathogenesis of osteoarthritis. J Rheumatol 1986; 13: 1126-60.
5. Kellgren JH, Moore R. Generalized osteoarthritis and heberden's nodes. Br Med J 1952; 1: 181-7.
6. Brown MF. Cartilage changes in osteoarthritis and rheumatoid arthritis. In Hughes S, McCarthy I, editors. Sciences basic to orthopaedics. London: WB Saunders Co Ltd; 1998: 156-67.
7. Yelin E. The economics of osteoarthritis. In: Brandt M, Doherty M, Lohmander LS, editors. Osteoarthritis. Oxford: Oxford Medical Publications; 1998: 23-30.
8. Lanes SF, Lanza LL, Radensky PW, Yood RA, Meenan RF, Walker AM, Dreyer NA. Resource utilization and cost of care for rheumatoid arthritis and osteoarthritis in a managed care setting: the importance of drug and surgery costs. Arthritis and Rheumatism 1997; 40(8): 1475-1481.
9. Kamelchuk LS, Major PW. Degenerative disease of the temporomandibular joint. J Orofacial Pain 1995; 9: 168-180.

10. Gangarosa LP, Mahan PE, Ciarlone AE. Pharmacologic management of temporomandibular joint disorders and chronic head and neck pain. J Cranio Practice 1991; 9: 328-38.

11. Gray RJM, Davies SJ, Quayle AA. A clinical approach to temporomandibular disorders. A clinical approach to treatment. Br Dent J 1994; 177: 101-06.

12. Agrawal NM. Anti-inflammatories and gastroduodenal damage: Therapeutic options. Eur J Rheum Inflam 1993; 13: 17-24.

13. Geis GS, Stead H, Wallemark CB, Nicholson PA. Prevalence of mucosal lesions in the stomach and duodenum due to chronic use of NSAID's in patients with rheumatoid arthritis or osteoarthritis, and interm report on prevention by misoprostol of diclofenac associated lesions. J Rheumatol 1991;18 Suppl 28: 11

14. de Pouvourville G, Tasch RF. The economic consequences of NSAID-induced gastrointestinal damage. Eur J Rheum Inflam 1993; 13: 33-40.

15. Simon LS. Biologic effects of nonsteroidal anti-inflammatory drugs. Curr Opin heumatol 1997; 9: 178-82.

16. Bandt KD. Should nonsteroidal anti-inflammatory drugs be used to treat osteoarthritis? Rheum Clin North Am 1993; 19: 29-44.

17. Brandt KD. Should osteoarthritis be treated with nonsteroidal anti-inflammatory drugs? Rheum Dis Clin North Am 1993; 19(3): 697-712.

18. Huskisson EC, Berry H, Gishen P, Jubb RW, Whitehead J. Effects of anti-inflammatory drugs on the progression of osteoarthritis of the knee. J Rheumatol 1995; 22: 1941-46.

19. Sheild MJ. Anti-inflammatory drugs and their effects on cartilage synthesis and renal function. Eur J Rheum Inflamm 1993; 13(1): 7-16.

20. Lehninger AL. Biochemistry. New York: Worth Publisher; 1975.

21. Bohne W. Glukosamine in der konservativen arthrosebehanlung. Med Welt 1969; 30: 1668-71.

22. Crolle G, D'Este E. Glucosamine sulphate for the management of arthrosis: a controlled clinical investigation. Curr Med Res Opin 1980; 7: 104-09.

23. Drovanti A, Bignamini AA, Rovati AL. Therapeutic activity or oral glucosamine sulphate in osteoarthrosis: a placebo-controlled double-blind investigation 1980; Clin Ther 3: 260-72.

24. D'Ambrosio E, Casa B, Bompani R. Glucosamine sulphate: a controlled clinical investigation in arthrosis. Pharmatherapeutica 1981; 2: 504-08.

25. Pujalte JM, Llavore EP, Ylescupidez FR. Double-blind clinical evaluation of oral glucosamine sulphate in the basic treatment of osteoarthrosis. Curr Med Res Opin 1980; 7: 110-14.

26. Setnikar I, Pacini MA, Revel L. Antiarthritic effects of glucosamine sulphate studied in animal models. Arzneim Forsch 1991; 41: 542-45.

27. Moriga M, Aona M, Murakami M, Uchino H. The activity of N-acetylglucosamine kinase in rat gastric mucosa. Gastroenterol Japonica 1980; 15: 7-13.

28. Reichelt A, Forster KK, Fischer M, Rovati LC, Setnikar I. Efficacy and safety of intramuscular glucosamine sulphate in osteoarthritis of the knee. Arzneim Forsch 1994; 44: 75-80.

29. Tapadinhas MJ, Rivera IC, Bignamini AA. Oral glucosamine sulphate in the management of arthrosis: report on a multi-centre open investigation in Portugal. Pharmatherapeutica 1982; 3: 157-68.

30. Buckwalter JA, Mankin HJ. Articular cartilage: Degeneration and osteoarthritis, repair, regeneration, and transplantation. AAOS instructional course lectures 1998; 47: 487-504.

31. Hough AJ. Pathology of osteoarthritis. In: Koopman WJ editor. Arthritis and allied conditions: A textbook of Rheumatology, 13th edition. Baltimore: Williams & Wilkins; 1997: 1945-67.

32. Hunziker EB, Rosenberg L. Articular cartilage repair. In: Koopman WJ editor. Arthritis and allied conditions: A textbook of Rheumatology, 13th edition. Baltimore: Williams & Wilkins; 1997: 2027-38.

33. Grinnell F. Fibroblasts, myofibroblasts, and wound contraction. J Cell Biol 1994; 124: 401-04.

34. Pelletier JP, Haraoui B, Fernandes JC. New and future therapies for osteoarthritis. In: Reginster JY, Pelletier JP, Martel-Pelletier J, Henrotin Y, editors. Osteoarthritis: Clinical and experimental aspects. Berlin: Springer; 1999: 387-408.

35. Mankin HJ. The reaction of articular cartilage to injury and osteoarthritis (first of two parts). N Engl J Med 1974; 291: 1285-92.

36. Altman RD, Kates J, Chun LE, Dean DD, Eyre D. Preliminary observations of chondral abrasion in a canine model. Ann Rheum Dis 1992; 51: 1056-62.

37. Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. J Bone Joint Surg 1976; 58: 230-33.

38. Shapiro F, Koide S, Glimcher MJ. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. J Bone Joint Surg 1993; 75A: 532-553.

39. Nimni M, Deshmukh K. Differences in collagen metabolism between normal and osteoarthritic human articular cartilage. Science 1973; 181: 751-52.

40. Adam M, Deyl Z. Altered expression of collagen phenoType In osteoarthrosis. Clin Chim Acta 1983; 133: 25-32.

41. Gay S, Muller PK, Lemmen C, Remberger K, Matzen K, Kuhn K. Immunohistological study on collagen in cartilage-bone metamorphosis and degenerative osteoarthrosis. Klin Wochenschr 1976; 54: 969-76.

42. Aigner T, Bertling W, Stoss H, Weseloh G, von der Mark K. Independent expression of fibril-forming collagens I, II, and III in chondrocytes of human osteoarthritic cartilage. J Clin Invest 1993; 91:829-37.

43. Aigner T, Dietz U, Stoss H, von der Mark K. Differential expression of collagen types I, II, III, and X in human osteophytes. Lab Invest 1995; 73:236-43.

44. Radin EL, Ehrlich MG, Chernack R, Abernethy P, Paul IL, Rose RM. Effect of repetitive impulsive loading on the knee joints of rabbits. Clin Orthop Rel Res 1978; 131:288-93.

45. Layton MW, Goldstein SA, Goulet RW, Feldkamp LA, Kubinski DJ, Bole GG. Examination of subchondral bone architecture in experimental osteoarthritis by microscopic computed axial tomography. Arthritis Rheum 1988; 31:1400-5.

46. Radin EL, Burr DB, Caterson B, Fyhrie D, Brown TD, Boyd RD. Mechanical determinants of osteoarthrosis[Review]. Sem Arthritis Rheum 1991; 21(3 Suppl 2):12-21.

47. Dedrick DK, Goldstein SA, Brandt KD, O'Connor BL, Goulet RW, Albrecht M. A longitudinal study of subchondral plate and trabecular bone in cruciate-deficient dogs with osteoarthritis followed up for 54 months. Arthritis Rheumat 1993; 36:1460-7.

48. Van den Berg WB, van der Kraan PM, van Beuningen HM. Synovial mediators of cartilage damage and repair in OA. In: Brandt M, Doherty M, Lohmander LS, editors. Osteoarthritis. Oxford: Oxford Medical Publications; 1998: 157-67.

49. Myers SL, Flusser D, Brandt KD, Heck DA. Prevalence of cartilage shards in synovium and their association with synovitis in patients with early and endstage osteoarthritis. J Rheum 1992; 19:1247-51.

50. Tyler JA. Insulin-like growth factor 1 can decrease degradation and promote synthesis of proteoglycan in cartilage exposed to cytokines. Biochem J 1989; 260:543-8.

51. Amin AR, Di Cesare PE, Vyas P, Attur M, Tzeng E, Billiar TR, Stuchin SA, Abramson SB. The expression and regulation of nitric oxide synthase in human osteoarthritis-affected chondrocytes: evidence for up-regulated neuronal nitric oxide synthase. J Exp Med 1995; 182:2097-102.

52. Blanco FJ, Ochs RL, Schwarz H, Lotz M. Chondrocyte apoptosis induced by nitric oxide. Am J Path 1995; 146:75-85.

53. Verschure PJ, Van Noorden CJ, Van Marle J, Van den Berg WB. Articular cartilage destruction in experimental inflammatory arthritis: insulin-like growth factor-1 regulation of proteoglycan metabolism in chondrocytes. Histochem J 1996; 28:835-57.

54. Towle CA. Han Hwa Hung. Bonassar LJ. Treadwell BV. Mangham DC. Detection of interleukin-1 in the cartilage of patients with osteoarthritis: A possible autocrine/paracrine role in pathogenesis. Osteoarthritis Cart 1997; 5: 293-300.

55. Chambers MG, Bayliss MT, Mason RM. Chondrocyte cytokine and growth factor expression in murine osteoarthritis. Osteoarthritis Cart 1997; 5: 301-08.

56. Webb GR, Westacott CI, Elson CJ. Chondrocyte tumor necrosis factor receptors and focal loss of cartilage in osteoarthritis. Osteoarthritis Cart. 1997; 5: 427-437.

57. Blanco FJ. Guitian R. Vazquez-Martul E. De Toro FJ. Galdo F. Osteoarthritis chondrocytes die by apoptosis: A possible pathway for osteoarthritis pathology. Arthritis & Rheum 1998; 41: 284-89.

58. Pelletier JM. Pathophysiology of osteoarthritis. Osteoarthritis and Cart 1999; 7: 371-73.

59. Pelletier JM. Proinflammatory mediators and osteoarthritis. Osteoarthritis and Cart 1999; 7: 315-16.

60. Pelletier JP, Martel-Pelletier J, Howell DS. Etiopathogenesis of osteoarthritis. In: Koopman WJ editor. Arthritis and allied conditions: A textbook of Rheumatology, 13th edition. Baltimore: Williams & Wilkins; 1997: 1969-84.

61. Pelletier JP, Mineau F, Ranger P, Tardif G, Martel-Pelletier J. The increased synthesis of inducible nitric oxide inhibits IL-1ra synthesis by human articular chondrocytes: possible role in osteoarthritic cartilage degradation. Osteoarthritis Cart 1996; 4:77-84.

62. Farrell AJ, Blake DR, Palmer RM, Moncada S. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann Rheum Dis 1992; 51:1219-22.

63. Lajeunesse D, Hilal G, Pelletier JP, Martel-Pelletier J. Subchondral bone morphological and biochemical alterations in osteoarthritis. Osteoarthritis and Cart 1999; 7: 321-22

64. Moskowitz RW. Bone remodeling in osteoarthritis: subchondral and osteophytic responses. Osteoarthritis and Cart 1999; 7: 323-24.

65. Peyron JG. Osteoarthritis. The epidemiologic viewpoint. In Ehrlich MG editor. Osteoarthritis. Philadelphia: Lippincott LB 1986: 13-19.

66. Felson DT. The epidemiology of osteoarthritis: Prevalence and risk factors. In: Keuttner KE, Goldberg VM, editors. Osteoarthritic disorders. American Academy of orthopedic surgeons, Rosemont;1995: 13-24.

67. Wiberg B, Wanman A. Signs of osteoarthrosis of the temporomandibular joints in young patients. A clinical and radiographic study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 86: 158-64.

68. Dijkgraaf LC, Liem RSB, de Bont LGM. Synovial membrane involvement in osteoarthritic temporomandibular joints. A light microscopic study. . Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997; 83: 373-86.

69. Bates RE, Gremillion HA, Stewart CM. Degenerative joint disease. Part I: Diagnosis and management considerations. J Cranimand Pract 1993; 11: 284-290.

70. Dibbets JMH, Weele van der Lt, Boering G. Craniofacial morphology and temporomandibular joint dysfunction in children. In: Carlson DS, McNamara JA, Ribbens KA editors. Development aspects of temporomandibular joint disorders monograph 16, craniofacial growth series. Centre for human growth and development Ann Arbor; The University of Michigan 1985: 151-182.

71. Toller PA. Osteoarthritis of the mandibular condyle. Brit Dent J 1973; 134: 223-231.

72. Ogus H. Degenerative disease of the temporomandibular joint and pain dysfunctionsyndrome. J Royal Soc Med 1978; 71: 748-754.

73 Toller PA. Temporomandibular arthropathy. Proc Royal Soc Med 1974; 67: 153-159.

74. Rasmussen OC. Temporomandibular arthropathy: Clinical, radiologic and therapeutic aspects with emphasis on diagnosis. Int J Or Surg 1983; 12: 365-397.

75. Berrett A. Radiography of the temporomandibular joint. Dent Clin North Am 1983; 27: 527-540.

76. Mejersjo C, Hollender L. Radiography of the temporomandibular joint in female patients with TMJ pain or dysfunction. Acta Radiol [Diag] 1984; 25: 169-176.

77. Magnusson T, Carlsson GE. Treatment of patients with functional disturbances of the masticatory system. A survey of 80 consecutive patients. Swed Dent J 1980; 4:145-153.

78. Poswillo D. Conservative management of degenerative temporomandibular joint disease in the elderly. Int Dent J 1983; 33: 325-331.

79. Hansson LG, Peterson A, Vallon-Christersson D. Clinical and radiographic six yearfollow-up study of patients with crepitation of the temporomandibular joint. Swed Dent J 1984: 8: 277-287.

80. Rasmussen OC. Clinical findings during the course of temporomandibular arthropathy. Scand J Dent Res 1981; 89: 283-288.

81. Lindvall AM, Helkimo E, Hollender L, Carlsson GE. Radiographic examination of the temporomandibular joints. A comparison between radiographic findings and gross microscopic morphologic observations. Dentomaxillofac Radiol 1976; 5: 24-32.

82. Carlsson GE, Lundberg M, Oberg T, Welander U. The temporomandibular joint. A comparative anatomic and radiologic study. Odont Revy 1968; 19: 171-185.

83. Sokoloff L. The biology of degenerative joint disease. Chicago: University of Chicago Press, 1969.

84. de Bont LGM, Boering G, Liem RSB, Havinga P. Osteoarthritis of the temporomandibular joint: A light microscope and scanning electronmicroscope of the articular cartilage of the mandibular condyle. J Oral and Maxillofacial Surg 1985; 43:481-488.

85. Benson BW, Otis LL. Disorders of the temporomandibular joint. Dent Clin N Am 1994; 38(1): 167-185.

86. Bates Jr RE, Gremillion HA, Stewart CM. Degenerative joint disease. Part I. Diagnosis and management considerations. J Craniomand Pract 1993; 11(4):84-290.

87. Kopp S, Rockler B. Relationship between clinical and radiographic findings in patients with mandibular pain or dysfunction. Acta Radiol [Diag] 1979; 20: 465-477.

88. Stegenga B, de Bont LGM, Boering G. Osteoarthritis as the cause of craniomandibular dysfunction: A unifying concept. J Oral Maxillofac Surg 1989; 47: 249-256.

89. Haskin CL, Milam SB, Cameron IL. Pathogenesis of degenerative joint disease in the human temporomandibular joint. Crit Rev Oral Biol Med 1995; 6: 248-277.

90. Oberg T, Carlsson GE, Fajers CM. The temporomandibular joint: A morphological study on human autopsy material. Acta Odontol Scand 1971; 29: 349-84.

91. Rohlin M, Westesson P-I, Ericksson L. The correlation of temporomandibular joint sounds with morphology in fifty-five autopsy specimens. J Oral Maxillofac Surg 1985; 43: 194-200.

92. Axelsson S, Fitins D, Hellsing G, Holmlund A. Arthrotic changes and deviation in form of the temporomandibular joint--an autopsy study. Swed Dent J 1987; 11:195-200.

93. Westesson PL. Rohlin M. Internal derangement related to osteoarthrosis in temporomandibular joint autopsy specimens. Oral Surg Oral Med Oral Path 1984 57:17-22.

94. Silbermann M, Livne E. Age-related degenerative changes in the mouse mandibular joint. J Anat 1979; 129: 507-20

95. Bouvier M. Effects of age on the ability of the rat temporomandibular joint to respond to changing functional demands. J Dent Res 1988; 67:1206-12.

96. Dreessen D, Halata Z. Age-related osteo-arthrotic degeneration of the temporomandibular joint in the mouse. Acta Anat 1990: 139: 91-6.

97. Livne E, von der Mark K, Silbermann M. Morphologic and cytochemical changes in maturing and osteoarthritic articular cartilage in the temporomandibular joint of mice. Arthritis Rheum 1985 28:1027-38.

98. Livne E, Weiss A, Silbermann M. Articular chondrocytes lose their proliferative activity with aging yet can be restimulated by PTH-(1-84), PGE1, and dexamethasone. J Bone & Miner Res 1989; 4:539-48.

99. Livne E. Weiss A. Silbermann M. Changes in growth patterns in mouse condylar cartilage associated with skeletal maturation and senescence. Growth Dev Aging 1990; 54:183-93.

100. de Bont LG. Liem RS. Boering G. Ultrastructure of the articular cartilage of the mandibular condyle: aging and degeneration. Oral Surg Oral Med Oral Path 1985 60:631-41.

101. Martin JA, Buckwalter JA. Articular cartilage aging and degeneration. Sports Med Arthrosc Rev 1996; 4: 263-75.

102. Buckwalter JA, Lane LE. Aging, sports, and osteoarthritis. . Sports Med Arthrosc Rev 1996; 4: 276-287.

103. Buckwalter JA, Lane NE, Gorden SL. Exercise as a cause of osteoarthritis. In: Keuttner KE, Goldberg VM, editors. Osteoarthritic disorders. American Academy of orthopedic surgeons, Rosemont; 1995: 405-17.

104. Pullinger AG. Seligman DA. Gombein JA. A multiple logistic regression analysis of the risk and relative odds of temporomandibular disorders as a function of common occlusal features. J Dent Res 1993; 72: 968-79.

105. Seligman DA. Pullinger AG. Association of occlusal variables among refined TM patient diagnostic groups. J Craniomand Dis 1989; 3:227-36.

106. Holmlund A. Hellsing G. Arthroscopy of the temporomandibular joint. A comparative study of arthroscopic and tomographic findings. Int J Oral Maxillofac Surg 1988; 17:128-33

107. Nebbe B. Adolescent TMJ and craniofacial morphology. PhD thesis University of Alberta; Edmonton.1998; 60.

108. Nickel JC, McLachlan KR. In vitro measurement of the frictional properties of the temporomandibular joint disc. Archs Oral Biol 1994; 39: 323-31.

109. Axelsson S. Holmlund A. Hjerpe A. Glycosaminoglycans in normal and osteoarthrotic human temporomandibular joint disks. Acta Odontol Scand 1992 50:113-9.

110. Mongini F. Influence of function on temporomandibular joint remodeling and degenerative disease. Dent Clin North Am 1983; 27:479-94.

111. Pullinger AG. Seligman DA. TMJ osteoarthrosis: a differentiation of diagnostic subgroups by symptom history and demographics. J of Craniomand Disorders 1987; 1: 251-6.

112. Maroudas A. Physiochemical properties of articular cartilage. Adult articular cartilage (Ed. Freeman MAR), 2nd edn, Pitman Medical, London 1979: 215-90.

113. Walker PS, Unsworth A, Dowson D, Sikorski J, Wright V. Mode of aggregation of hyaluronic acid protein complex on the surface of articular cartilage. Ann Rheum Dis 1970; 29:591-602.

114. Hills BA. Oligolamellar lubrication of joints by surface active phospholipid. J Rheum 1989 16:82-91.

115. Ghadially FN. Synovial membrane In: Ghadially FN editor. Fine structure of synovial joints. A text and atlas of the ultrastructure of normal and pathological articular tissues. London, Buttersworth; 1983: 1-41.

116. Kircos LT, Ortendahl DA, Mark AS, Arakawa M. Magnetic resonance imaging of the TMJ disc in asymptomatic volunteers. J Oral Maxillofac Surg 1987; 45:852-4.

117. Ribeiro RF, Tallents RH, Katzberg RW, Murphy WC, Moss ME, Magalhaes AC, Tavano O. The prevalence of disc displacement in symptomatic and asymptomatic volunteers aged 6 to 25 years. J Orofacial Pain 1997; 11:37-47.

118. Katzberg RW, Westesson PL, Tallents RH, Drake CM. Orthodontics and temporomandibular joint internal derangement. Am J Orthod Dentofac Orthop 1996; 109:515-20.

119. Scapino RP. Histopathology associated with malposition of the human temporomandibular joint disc. Oral Surg Oral Med Oral Pathol 1983; 55: 382-97.

120. Ericsson L, Westesson PL. Long-term evaluation of meniscectomy of the temporomandibular joint. J Oral Maxillofac Surg 1985; 43: 263-69.

121. Westesson PL. Structural hard-tissue changes in the temporomandibular joints with internal derangement. Oral Surg Oral Med Oral Pathol 1985; 59:220-24.

122. Cholitgul W, Petersson A, Rohlin M, Akerman S. Clinical and radiological findings in temporomandibular joints with disc perforation. Int J Oral Maxillofac Surg 1990; 19:220-5.

123 Helmy ES, Bays RA, Sharawy MM. Histopathological study of human TMJ perforated discs with emphasis on synovial membrane response. J Oral Maxillofac Surg 1989; 47: 1048-1052.

124. Helmy ES. Light microscopic and ultrastructural study of thinned discal areas in patients with temporomandibular joint internal derangement. Egyptian Dent J 1993; 39:325-36.

125. Anderson QN. Katzberg RW. Pathologic evaluation of disc dysfunction and osseous abnormalities of the temporomandibular joint. J Oral Maxillofac Surg 1985 43:947-51.

126. Brand JW, Whinery JG Jr, Anderson QN, Keenan KM. The effects of temporomandibular joint internal derangement and degenerative joint disease on tomographic and arthrotomographic images. Oral Surg Oral Med Oral Pathol 1989 67:220-3.

127. Stegenga B, de Bont LG, Boering G. Osteoarthrosis as the cause of craniomandibular pain and dysfunction: a unifying concept. J Oral Maxillofac Surg 1989 47:249-56.

128. Mankin HJ, Radin EL. Structure and function of joints. In: Koopman WJ editor. Arthritis and allied conditions: A textbook of Rheumatology, 13th edition. Baltimore: Williams & Wilkins; 1997: 175-191.

129. O'Rahilly R, Gardner E. The embryology of movable joints. In: Sokoloff L, editior. The joints and synovial fluid. New York; Academic Press: 1978: 49-103.

130. Wheater PR, Burkitt HG, Daniels VG. Functional Histology. Edinburgh; Churchill and Livingstone: 1979.

131. Hylander WL. Functional Anatomy In: Sarnat BG, Laskin DM editors.The temporomandibular joint. Philadelphia; WB Saunders; 1992: 60-92.

132. Cate ART. Gross and micro anatomy. In: Zarb GA, Carlsson GE, Sessle BJ, Mohl ND editors. Temporomandibular joint and masticatory muscle disorders. Copenhagen, Munksgaard, 2nd ed. 1994; 48-66.

133. Thonar EJ-MA, Masuda K, Manicourt DH, Kuettner KE. Structure and function of normal human adult articular cartilage. In: Reginster JY, Pelletier JP, Martel-Pelletier J, Henrotin Y, editors. Osteoarthritis: Clinical and experimental aspects. Berlin: Springer; 1999: 1-19.

134. Dijkgraaf LC, de Bont LGM, Boering G, Liem RSB. Normal cartilage structure, biochemistry, and metabolism: A review of the literature. J Oral Maxillofac Surg 53: 924-29.

135. Hamerman D. The biology of osteoarthritis. N Engl J Med 1989; 320: 1322-30.

136. de Bont LG. de Haan P. Boering G. Structure and growth of the cartilage of the temporomandibular joint. Nederlands Tijdschrift voor Tandheelkunde. 1985; 92:184-9.

137. Aydelotte MB, Kuettner KE. Differences between sub-populations of cultured bovine articular chondrocytes. I. Morphology and cartilage matrix production. Connect Tiss Res 1988 18:205-22.

138. de Bont LG. Boering G. Havinga P. Liem RS. Spatial arrangement of collagen fibrils in the articular cartilage of the mandibular condyle: a light microscopic and scanning electron microscopic study. J Oral Maxillofac Surg 1984; 42:306-13.

139. Hunziker EB. Articular cartilage structure in humans and experimental animals. In: Kuettner KE, Schleyerbach R, Peyron J, Hascall VC editors. Articular cartilage and osteoarthritis. New York, Raven Press; 1992: 183-99

140. Eyre DR, Wu JJ, Woods P. The cartilage-specific collagens: Structural studies. In: Kuettner KE, Schleyerbach R, Peyron J, Hascall VC editors. Articular cartilage and osteoarthritis. New York, Raven Press; 1992: 119-31.

141. Eyre DR, Wu JJ, Niyibizi C, Chun L. The cartilage collagens: Analysis of their cross-linking interactions and matrix organization. In: Maroudas A, Kuettner K editors. Methods in cartilage research. London, Academic Press; 1990: 28-33.

142. Ostergaard K, Salter DM. Immunohistochemistry in the study of normal and osteoarthritic articular cartilage. Progr Histochem Cytochem 1998; 33: 93-168.

143. Kraus VB. Pathogenesis and treatment of osteoarthritis. Med Clinics North Am 1997; 81: 85-112.

144. Hardingham TE, Fosang AJ, Dudhia J. Aggrecan, the chondroitin sulphate/keratan sulphate proteoglycan form cartilage. In: Kuettner KE, Schleyerbach R, Peyron J, Hascall VC editors. Articular cartilage and osteoarthritis. New York, Raven Press; 1992: 5-20.

145. Witsch-Prehm P. Miehlke R. Kresse H. Presence of small proteoglycan fragments in normal and arthritic human cartilage. Arthritis Rheum 1992 35:1042-52.

146. Dijkgraaf LC, De Bont LG, Boering G, Liem RS. Function, biochemistry, and metabolism of the normal synovial membrane of the temporomandibular joint: a review of the literature. J Oral Maxillofac Surgery. 1996; 54:95-100.

147. de Bont LG, Liem RS, Boering G. Ultrastructure of the articular cartilage of the mandibular condyle: aging and degeneration. Oral Surg Oral MedOral Pathol 1985; 60:631-41.

148. Quinn JH. Arthroscopic and histologic evidence of chondromalacia in the temporomandibular joint. Oral Surg Oral Med Oral Path 1990; 70:387-92.

149. de Bont LG, Boering G, Liem RS, Havinga P. Osteoarthritis of the temporomandibular joint: a light microscopic and scanning electron microscopic study of the articular cartilage of the mandibular condyle. J Oral Maxillofac Surg 1985;43:481-8.

150. de Bont LG. Boering G. Liem RS. Eulderink F. Westesson PL. Osteoarthritis and internal derangement of the temporomandibular joint: a light microscopic study. J Oral Maxillofac Surg 1986; 44:634-43.

151. Dijkgraaf LC, de Bont LG, Boering G, Liem RS. The structure, biochemistry, and metabolism of osteoarthritic cartilage: a review of the literature. J Oral Maxillofac Surg 1995; 53:1182-92.

152. Ghadially FN. Synovial membrane In: Ghadially FN, editor. Fine structure of synovial joints. A text and atlas of the ultrastructure of normal and pathologic articular tissues. London; Butterworths; 1983: 1-41.

153. Blau S, Janis R, Hamerman D, Sandson J. Cellular origin of hyaluronate protein in the human synovial membrane. Science 1965; 150: 353.

154. Israel HA, Saed-Nejad F, Ratcliffe A. Early diagnosis of osteoarthrosis of the temporomandibular joint: correlation between arthroscopic diagnosis and keratan sulfate levels in the synovial fluid. J Oral Maxillofac Surg 1991 49(7):708-11.

155. Israel HA, Diamond BE, Saed-Nejad F, Ratcliffe A. Correlation between arthroscopic diagnosis of osteoarthritis and synovitis of the human temporomandibular joint and keratan sulfate levels in the synovial fluid. J Oral Maxillofac Surg 1997; 55:210-7.

156. Shibata T, Murakami KI, Kubota E, Maeda H. Glycosaminoglycan components in temporomandibular joint synovial fluid as markers of joint pathology. J Oral Maxillofac Surg 1998 56:209-13.

157. Dijkgraaf LC, Liem RS, de Bont LG. Ultrastructural characteristics of the synovial membrane in osteoarthritic temporomandibular joints. J Oral Maxillofac Surg 1997; 55:1269-79.

158. Holmlund A, Hellsing G, Axelsson S. The temporomandibular joint: a comparison of clinical and arthroscopic findings. J Prosth Dent. 1989; 62:61-5.

159. Israel HA, Diamond B, Saed-Nejad F, Ratcliffe A. Osteoarthritis and synovitis as major pathoses of the temporomandibular joint: comparison of clinical diagnosis with arthroscopic morphology. J Oral Maxillofac Surg 1998; 56:1023-7.

160. Holmlund A, Hellsing G. Arthroscopy of the temporomandibular joint: occurrence and location of osteoarthrosis and synovitis in a patient material. Int J Oral Maxillofac Surg 1988; 17:36-40.

161. Dijkgraaf LC, Spijkervet FK, de Bont LG. Arthroscopic findings in osteoarthritic temporomandibular joints. J Oral Maxillofac Surg 1999; 57:255-68.

162. Kopp S. The influence of neuropeptides, serotonin, and interleukin 1beta on temporomandibular joint pain and inflammation. J Oral Maxillofac Surg 1998; 56:189-91.

163. Fu K. Ma X. Zhang Z. Chen W. Tumor necrosis factor in synovial fluid of patients with temporomandibular disorders. J Oral Maxillofac Surg. 1995; 53:424-6.

164. Kubota E, Imamura H, Kubota T, Shibata T, Murakami K. Interleukin 1 beta and stromelysin (MMP3) activity of synovial fluid as possible markers of osteoarthritis in the temporomandibular joint. J Oral Maxillofac Surg 1997; 55:20-7.

165. Kubota E. Kubota T. Matsumoto J. Shibata T. Murakami KI. Synovial fluid cytokines and proteinases as markers of temporomandibular joint disease: J Oral Maxillofac Surg 1998; 56:192-8.

166. Fang PK, Ma XC, Ma DL, Fu KY. Determination of interleukin-1 receptor antagonist, interleukin-10, and transforming growth factor-beta1 in synovial fluid aspirates of patients with temporomandibular disorders. J Oral Maxillofac Surg. 1999; 57:922-8.

167. Sandler NA, Buckley MJ, Cillo JE, Braun TW. Correlation of inflammatory cytokines with arthroscopic findings in patients with temporomandibular joint internal derangements. J Oral Maxillofac Surg 1998 56:534-43.

168. Takahashi T, Kondoh T, Kamei K, Seki H, Fukuda M, Nagai H, Takano H, Yamazaki Y. Elevated levels of nitric oxide in synovial fluid from patients with temporomandibular disorders. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996; 82:505-9.

169. Takahashi T, Kondoh T, Ohtani M, Homma H, Fukuda M. Association between arthroscopic diagnosis of temporomandibular joint osteoarthritis and synovial fluid nitric oxide levels. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999; 88:129-36.

170. Stegenga B, de Bont LG, Boering G, van Willigen JD. Tissue responses to degenerative changes in the temporomandibular joint: a review. J Oral Maxillofac Surg 1991; 49:1079-88.

171. de Bont LG, Stegenga B. Pathology of temporomandibular joint internal derangement and osteoarthrosis. Int J Oral Maxillofac Surg 1993; 22:71-4.

172. Milam SB, Schmitz JP. Molecular biology of temporomandibular joint disorders: proposed mechanisms of disease. J Oral Maxillofac Surg 1995; 53:1448-54.

173. Milam SB, Zardeneta G, Schmitz JP. Oxidative stress and degenerative temporomandibular joint disease: a proposed hypothesis. J Oral Maxillofac Surg 1998; 56:214-23.

174. Felson DT. Epidemiology of osteoarthritis. In: Brandt KD, Doherty M, Lohmander LS, editors. Osteoarthritis. Oxford: Oxford University Press; 1998: 13-22.

175. Felson DT, Naimark A, Anderson J, Kazis L, Castelli W, Meenan RF. The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. Arthrit Rheumat 1987; 30:914-8.

176. van Saase JL. van Romunde LK. Cats A. Vandenbroucke JP. Valkenburg HA. Epidemiology of osteoarthritis: Zoetermeer survey. Comparison of radiological osteoarthritis in a Dutch population with that in 10 other populations. Ann Rheumat Dis 1989; 48:271-80.

177. Eriksen EF, Colvard DS, Berg NJ, Graham ML, Mann KG, Spelsberg TC, Riggs BL. Evidence of estrogen receptors in normal human osteoblast-like cells. Science. 1988; 241:84-6.

178. Komm BS, Terpening CM, Benz DJ, Graeme KA, Gallegos A, Korc M, Greene GL, O'Malley BW, Haussler MR. Estrogen binding, receptor mRNA, and biologic response in osteoblast-like osteosarcoma cells. Science 1988; 241:81-4.

179. Turner RT, Riggs BL, Spelsberg TC. Skeletal effects of estrogen. Endocr Rev 1994; 15:275-300.

180. Vedi S. Compston JE. The effects of long-term hormone replacement therapy on bone remodeling in postmenopausal women. Bone 1996; 19:535-9.

181. Turner AS, Athanasiou KA, Zhu CF, [illegible]is MR, Bryant HU. Biochemical effects of estrogen on articular cartilage in ovariectomized sheep. Osteoarthritis Cart 1997; 5:63-9.

182. Rosner IA, Malemud CJ, Hassid AI, Goldberg VM, Boja BA, Moskowitz RW. Estradiol and tamoxifen stimulation of lapine articular chondrocyte prostaglandin synthesis. Prostaglandins 1983; 26:123-38.

183. Tsai CL, Liu TK. Up-regulation of estrogen receptors in rabbit osteoarthritic cartilage. Life Sci 1992; 50:1727-35.

184. Rosner IA, Goldberg VM, Getzy L, Moskowitz RW. Effects of estrogen on cartilage and experimentally induced osteoarthritis. Arthritis Rheumat 1979; 22:52-8.

185. De M, Sanford TR, Wood GW. Interleukin-1, interleukin-6, and tumor necrosis factor alpha are produced in the mouse uterus during the estrous cycle and are induced by estrogen and progesterone. Dev Biol 151:297-305.

186. Cutolo M, Accardo S, Villaggio B, Clerico P, Bagnasco M, Coviello DA, Carruba G, Io Casto M, Castagnetta L. Presence of estrogen-binding sites on macrophage-like synoviocytes and CD8+, CD29+, CD45RO+ T lymphocytes in normal and rheumatoid synovium. Arthritis Rheumat 1993; 36:1087-97.

187. Felson DT, Nevitt MC. Estrogen and osteoarthritis: how do we explain conflicting study results? Preventive Med. 1999;28:445-8.

188. Von Korff M, Dworkin SF, Le Resche L, Kruger A. An epidemiologic comparison of pain complaints. Pain 1988;32:173-83.

189. Dworkin SF, Huggins KH, LeResche L, Von Korff M, Howard J, Truelove E, Sommers E. Epidemiology of signs and symptoms in temporomandibular disorders: clinical signs in cases and controls. J Am Dent Ass 1990; 120:273-81.

190. Locker D. Slade G. Prevalence of symptoms associated with temporomandibular disorders in a Canadian population. Community Dent Oral Epidemiol 1988; 16:310-3.

191. LeResche L, Saunders K, Von Korff MR, Barlow W, Dworkin SF. Use of exogenous hormones and risk of temporomandibular disorder pain. Pain 1997; 69:153-60.

192. Aufdemorte TB, Van Sickels JE, Dolwick MF, Sheridan PJ, Holt GR, Aragon SB, Gates GA. Estrogen receptors in the temporomandibular joint of the baboon (Papio cynocephalus): an autoradiographic study. Oral Surg Oral Med Oral Pathol 1986; 61:307-14.

193. Ng MC, Harper RP, Le CT, Wong BS. Effects of estrogen on the condylar cartilage of the rat mandible in organ culture. J Oral Maxillofac Surg 1999; 57:818-23.

194. Milam SB, Aufdemorte TB, Sheridan PJ, Triplett RG, Van Sickels JE, Holt GR. Sexual dimorphism in the distribution of estrogen receptors in the temporomandibular joint complex of the baboon. Oral Surg Oral Med Oral Pathol 1987; 64:527-32.

195. Gangarosa LP, Mahan PE, Ciarlone AE. Pharmacologic management of temporomandibular joint disorders and chronic head and neck pain. J Cranio Practice 1991; 9: 328-38.

196. Gray RJM, Davies SJ, Quayle AA. A clinical approach to temporomandibular disorders. A clinical approach to treatment. Br Dent J 1994; 177: 101-06.

197. Schnitzer TJ. Osteoarthritis treatment update. Minimizing pain while limiting patient risk. Postgrad Med. 1993 93:89-92.

198 Agus B, Weisberg J, Friedman MH. Therapeutic injection of the temporomandibular joint. Oral Surg Oral Med Oral Pathol 1983; 55:553-5.

199. Poswillo D. Experimental investigation of the effects of intra-articular hydrocortisone with high condylectomy of the mandibular condyle. Oral Surg 1970; 30: 161-173.

200. Fader KW, Grummons DC, Maijer R, Christensen LV. Pressurized infusion of sodium hyaluronate for closed lock of the temporomandibular joint. Part I: A case study. J Craniomand Pract 1993; 11:68-72.

201. Sato S, Sakamoto M, Kawamura H, Motegi K. Disc position and morphology in patients with nonreducing disc displacement treated by injection of sodium hyaluronate. Int J Oral Maxillofac Surg 1999 28:253-7.

202. Bertolami CN, Gay T, Clark GT, Rendell J, Shetty V, Liu C, Swann DA. Use of sodium hyaluronate in treating temporomandibular joint disorders: a randomized, double-blind, placebo-controlled clinical trial. J Oral Maxillofac Surg 1993; 51:232-42.

203. de Bont LGM, Dijkgraaf LC, Stegenga B. Epidemiology and natural progression of articular temporomandibular disorders. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997 83:72-6.

204. Frost DE, Kendell BD. Part II: The use of arthrocentesis for treatment of temporomandibular joint disorders. J Oral Maxillofac Surg 1999 57:583-7.

205. Wright V. Historical overview of NSAID's. Euro J Rheum Inflam 1994; 13 (1): 4-6.

206. Roth SH. Aspirin, salicylates, and nonsteroidal antiinflammatory drugs: New class labeling and medical practice. Comp Ther1991; 17: 15-21.

207. Brouwers JRBJ and deSmet PAGM. Pharmacokinetic-pharmacodynamic drug interactions with nonsteroidal anti-inflammatory drugs. Clin Pharmacokinet 1994; 27(6): 462-485.

208. Jouzeau JY. Terlain B. Abid A. Nedelec E. Netter P. Cyclo-oxygenase Isoenzymes. How recent findings affect thinking about nonsteroidal anti-inflammatory drugs. Drugs. 1997; 53:563-82.

209. Polisson R. Nonsteroidal anti-inflammatory drugs: practical and theoretical considerations in their selection. Am J Med 100 1996; (suppl2A):31S-36S.

210. Friedman SL. Practical classification for the use of nonsteroidal anti-inflammatory drugs. Journal Am Pod Med Ass 1996; 86:263-5.

211. Bellamy N. Treating musculoskeletal disease with NSAID's. Can Fam Physician 1996; 42: 482-92.

212. Day RO, Graham GG, Williams KM. Pharmacokinetics of non-steroidal anti-inflammatory drugs. Bailliere's Clin Rheum 1988; 2(2): 363-393.

213. Brune K, Graft P. Non-steroid anti-inflammatory drugs: influence of extra-cellular pH on biodistribution and pharmacological effects. Biochemical Pharmacol 1978 27:525-30.

214. Vane JR, Botting RM. Mechanism of action of aspirin-like drugs. Semin Arthritis Rheum 1997; 26: 2-10.

215. Smith WL, Dewitt DL. Prostaglandin endoperoxide H synthases-1 and -2. Adv Immunol 1996; 62:167-215.

216. Mandell BF. COX 2-selective NSAID's: biology, promises, and concerns. Cleveland Clinic J Med 1999 66:285-92.

217. Simon LS. Actions and toxicity of nonsteroidal anti-inflammatory drugs. Curr Opin Rheumatol 1995; 7: 159-166.

218. Cashman JN. The mechanisms of action of NSAID's in analgesia. Drugs 1996; 52 suppl (5): 13-23.

219. Simon LS. Biologic effects of nonsteroidal anti-inflammatory drugs. Curr Opin Rheumatol 1997; 9: 178-182.

220. Catania A, Arnold J, Macaluso A, Hiltz ME, Lipton JM. Inhibition of acute inflammation in the periphery by central action of salicylates. Proc Nat Acad Sci United States Am 1991 88:8544-7.

221. Urquhart E. Central analgesic activity of nonsteroidal antiinflammatory drugs in animal and human pain models. Sem Arthritis Rheumat 1993; 23:198-205.

222. Taiwo YO, Levine JD. Prostaglandins inhibit endogenous pain control mechanisms by blocking transmission at spinal noradrenergic synapses. J Neuroscience 1988 8:1346-49.

223. Malmberg AB, Yaksh TL. Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. Science 1992; 257:1276-79.

224. Malmberg AB, Yaksh TL. Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the formalin test in the rat. J Pharmacol Exp Therap 1992; 263:136-46.

225. Brune K, Beck WS, Geisslinger G, Menzel-Soglowek S, Peskar BM, Peskar BA. Aspirin-like drugs may block pain independently of prostaglandin synthesis inhibition. Experientia 1991 47:257-61.

226. Okuyama S, Aihara H. Inhibition of electrically-induced vocalization in adjuvant arthritic rats as a novel method for evaluating analgesic drugs. Jpn J Pharmacol 1984; 34:67-77.

227. Okuyama S. Aihara H. The mode of action of analgesic drugs in adjuvant arthritic rats as an experimental model of chronic inflammatory pain: possible central analgesic action of acidic nonsteroidal antiinflammatory drugs. Jpn J Pharmacol 1984; 35:95-103.

228. Willer JC. Comparative study of perceived pain and nociceptive flexion reflex in man. Pain 1977; 3:69-80.

229. Guieu R, Blin O, Pouget J, Serratrice G. Analgesic effect of indomethacin shown using the nociceptive flexion reflex in humans. Ann Rheum Dis 1992; 51:391-3.

230. Sandrini G, Ruiz L, Capararo M, Garofoli F, Beretta A, Nappi G. Central analgesic activity of ibuprofen. A neurophysiological study in humans. Int J Clin Pharmacol Res 1992 12:197-204.

231. Willer JC, De Broucker T, Bussel B, Roby-Brami A, Harrewyn JM. Central analgesic effect of ketoprofen in humans: electrophysiological evidence for a supraspinal mechanism in a double-blind and cross-over study. Pain 1989; 38:1-7.

232. Al Arfag A, Davis P. Osteoarthritis: current drug treatment regimens. Drugs 1991; 41: 193-201.

233. Brandt KD. Should nonsteroidal anti-inflammatory drugs be used to treat osteoarthritis ? Rheum Dis Clin North Am 1993; 19(1): 29-44.

234. Brandt KD. Should osteoarthritis be treated with nonsteroidal anti-inflammatory drugs? Rheum Dis Clin North Am 1993; 19(3): 697-712.

235. Fenner H. Differentiating among nonsteroidal antiinflammatory drugs by pharmacokinetic and pharmacodynamic profiles. Sem Arthritis & Rheum 1997; 26(6 Suppl 1):28-33.

236. Hollander D. Gastrointestinal complications of nonsteroidal anti-inflammatory drugs: prophylactic and therapeutic strategies. Am J Med 1994; 96: 274-281.

237. Clive DM, Stoff JS. Renal syndomes associated with nonsteroidal antiinflammatory drugs. N Engl J Med 1984; 310: 563-572.

238. Coles LS, Fries JF, Kraines RG. From experiment to experience: Side effects od nonsteroidal anti-inflammatory drugs. Am J Med 1983; 74: 820-828.

239. Griffin MR, Piper JM, Daugherty JR. Nonsteroidal anti-inflammatory drug use and increased risk for peptic ulcer disease in elderly persons. Ann Intern Med 1991;114: 257-263.

240. Lanza FL. Endoscopic studies of gastric and duodenal injury after use of ibuprofen, aspirin and other nonsteroidal anti-inflammatory drugs. Am J Med 77(suppl 1A)1984: 19-24.

241. Soll AH, Aeinstein WM, Kurata J. Nonsteroidal antiinflammatory drugs and peptic ulcer disease. Ann Intern Med 1991; 114: 307-319.

242. Wollheim FA. Current pharmacological treatment of osteoarthritis. Drugs 1996; 52 (suppl 3): 27-38.

243. Fries JF, Miller SR, Spitz PW, Williams CA, Hubert HB, Bloch DA. Toward an epidemiology of gastropathy associated with nonsteroidal antiinflammatory drug use. Gastroenterology 1989; 96(2 Pt 2 Suppl):647-55.

244. Guess HA, West R, Strand LM. Fatal upper gastrointestinal hemmorage or perforation among users and nonusers of nonsteroidal anti-inflammatory drugs in Saskatchewan, Canada. J Clin Epidemiol 1983; 41: 35-45.

245. Griffin MR, Ray WA, Schaffner W. Nonsteroidal anti-inflammatory drug use and death from peptic ulcer in elderly persons. Ann Intern Med 1988; 109:359-63.

246. Roth S, Agrawal N, Mahowald M, Montoya H, Robbins D, Miller S, Nutting E, Woods E, Crager M, Nissen C. Misoprostol heals gastroduodenal injury in patients with rheumatoid arthritis receiving aspirin. Arch Intern Med 1989; 149:775-9.

247. Armstrong CP, Blower AL. Nonsteroidal anti-inflammatory drugs and life threatening complications of peptic ulceration. Gut 1987; 28; 527-32.

248. Ray WA, Griffin MR, Shorr RI. Adverse drug reactions and the elderly. Health Affairs 1990 9:114-22.

249. Golden BD, Abramson SB. Selective cyclooxygenase-2 inhibitors. Rheum Dis Clin North Am 1999; 25:359-78.

250. Simon LS, Lanza FL, Lipsky PE, Hubbard RC, Talwalker S, Schwartz BD, Isakson PC, Geis GS. Preliminary study of the safety and efficacy of SC-58635, a novel cyclooxygenase 2 inhibitor: efficacy and safety in two placebo-controlled trials in osteoarthritis and rheumatoid arthritis, and studies of gastrointestinal and platelet effects. Arthritis Rheum 1998; 41:1591-602.

251. Fung HB. Kirschenbaum HL. Selective cyclooxygenase-2 inhibitors for the treatment of arthritis. Clin Therapeut 1999; 21:1131-57.

252. Brandt KD, Palmoski MJ. The effects of salicylates and other nonsteroidal anti-inflammatory drugs on articular cartilage. Am J Med 1984; 77: 65-69.

253. Palmoski MJ, Brandt KD. Effect of salicylate on proteoglycan metabolism in normal canine articular cartilage in vitro. Arthritis Rheum 1979; 22: 746-754.

254. Palmoski MJ, Brandt KD. Effects of some nonsteroidal anti-inflammatory drugs on proteoglycan metabolism and organization in canine articular cartilage. Arthritis Rheum 1980; 23: 1010-1020.

255. Brandt KD, Albrecht ME. Effect of naproxen sodium on the net synthesis of glycosaminoglycans and protein by normal canine articular cartilage in vitro. J Pharmacol 1990; 42: 738-40.

256. Brandt KD, Albrecht ME, Kalasinski LA. Effects of tiaprofenic acid on the concentration and metabolism of proteoglycans in normal and degenerating canine articular cartilage. J Clin Pharmacol 1990; 30: 808-14.

257. Palmoski MJ, Brandt KD. Benoxaprofen stimulates proteoglycan synthesis in normal canine knee cartilage in vitro. Arthritis Rheum 1983; 26: 771-74.

258. Dingle JT. Non-steroidal, anti-inflammatory drug administration in the treatment of osteoarthritis. In: Reginster JY, Pelletier JP, Martel-Pelletier J, Henrotin Y, editors. Osteoarthritis: Clinical and experimental aspects. Berlin: Springer; 1999: 370-87.

259. Palmoski MJ, Brandt KD. In vivo effect of aspirin on canine osteoarthritic cartilage Arthritis Rheum 1993; 26: 994-1001.

260. Bassleer C, Henrotin Y, Franchimont P. In-vitro evaluation of drugs proposed as chondroprotective agents. Int J Tiss Reac 1992; 14 (5): 231-41.

261. Setnikar I, Giacchetti C, Zanolo G. Pharmacokinetics of Glucosamine in the dog and man. Arzneim Forsch 1986; 36(1): 729-735.

262. Setnikar I, Palumbo R, Canali S, Zanolo G. Pharmacokinetics of glucosamine in man 1993; 43(10): 1109-13.

263. Roden L. Effect of hexosamine on the synthesis of chondroitin sulphuric acid in vitro. Ark Kemi 1956; 10: 345-52.

264. Karzel K. Domenjoz R. Effects of hexosamine derivatives and uronic acid derivatives on glycosaminoglycane metabolism of fibroblast cultures. Pharmacology. 1971; 5: 337-45.

265. Vidal y Plana RR, Bizzarri D, Rovati AL. Articular cartilage pharmacology: I. In vitro studies on glucosamine and nonsteroidal antiinflammatory drugs. Pharmacol Res Comm 1978 10:557-69.

266. Vidal y Plana RR, Karzel K. Glucosamine: its importance for the metabolism of articular cartilage. 2. Studies on articular cartilage. Fortschritte der Medizin 1980; 98: 801-6.

267. Bassleer C, Rovati L, Franchimont P. Stimulation of proteoglycan production by glucosamine sulphate in chondrocytes isolated from human osteoarthritic articular cartilage in vitro. Osteoarthritis Cart 1998; 6: 427-34.

268. Setnikar I, Cereda M, Pacini MA, Revel L. Antireactive properties of glucosamine sulphate. Arzneim Forsch 1991a; 41: 157-161.

269. Setnikar I, Pacini MA, Revel L. Antiarthritic effects of glucosamine sulphate studied in animal models. Arzneim Forsch 1991b; 41: 542-545.

270. Theodosakis J, Adderly B, Fox B. The Arthritis Cure. New York: St. Martin's Press. 1997.

271. Theodosakis J, Adderly B, Fox B. Maximizing the Arthritis Cure. New York: St. Martin's Press. 1998.

272. McCarty MF. The neglect of glucosamine as a treatment for osteoarthritis-a personal perspective. Medical Hypothesis 1994; 42: 323-27.

273. Bohne W. Glukosamine in der konservativen arthrosebehanlung. Med Welt 1969; 30: 1668-71.

274. Eichler J, Noh E. Therapy of deforming arthrosis through the action upon the cartilaginous metabolism. Orthop Praxis 1970; 6: 225-29.

275. Dustmann HO, Puhl W. Die intraartikulare injektionstherapie der arthrose-klinische und tierexperimentelle untersuchungen. Orthop Praxis 1978; 14: 682-84.

276. Mund-Hoym WD. Die behandlung von Huft and Kniegelenkarthrosen. Z Allg Med 1980; 56: 2153-2159.

277. Crolle G, D'Este E. Glucosamine sulphate for the management of arthrosis: a controlled clinical investigation. Curr Med Res Opin 1980; 7: 104-09.

278. Drovanti A, Bignamini AA, Rovati AL. Therapeutic activity or oral glucosamine sulphate in osteoarthrosis: a placebo-controlled double-blind investigation 1980; Clin Ther 3: 260-72.

279. D'Ambrosio E, Casa B, Bompani R. Glucosamine sulphate: a controlled clinical investigation in arthrosis. Pharmatherapeutica 1981; 2: 504-08.

280. Pujalte JM, Llavore EP, Ylescupidez FR. Double-blind clinical evaluation of oral glucosamine sulphate in the basic treatment of osteoarthrosis. Curr Med Res Opin 1980; 7: 110-14.

281. Vas AL. Double-blind clinical evaluation of the relative efficacy of ibuprofen and glucosamine sulphate in the management of osteoarthrosis of the knee in out-patients. Curr Med Opin 1980; 8: 145-149.

282 Barclay TS, Tsourounis C, McCart GM. Glucosamine. Ann Pharmacother 1998; 32: 574-79.

283. da Camara CC, and Dowless GV. Glucosamine sulphate for osteoarthritis. Ann Pharmacother 1998; 32: 580-87.

284. Deal CL and Moskowitz RW. Nutraceuticals as therapeutic agents in osteoarthritis: The role of glucosamine, chondroitin sulphate and collagen hydrolysate. Rheum Disease Clinics North Am 1999: 25: 379-395.

285. Muller-Fassbender H, Bach GL, Haase W, Rovati LC, Setnikar I. Glucosamine sulphate compared to ibuprofen in osteoarthritis of the knee. Osteoarthritis Cart 1994; 2: 61-69.

286. Qiu GX, Gao SN, Giacovelli G, Rovati L, Setnikar I. Efficacy and safety of glucosamine sulphate versus ibuprofen in patients with knee osteoarthritis. Arzneim-Forsch/Drug Res 1998; 48: 469-474.

287. Noack W, Fischer M, Forster KK, Rovati LC, Setnikar I. Glucosamine sulphate in osteoarthritis of the knee. Osteoarthritis Cart 1994; 2: 51-59.

288. Reichelt A, Forster RR, Fischer M, Rovati L, Setnikar I. Efficacy and safety of intramuscular glucosamine sulphate in osteoarthritis of the knee. Arzneim-Forsch/Drug Res 1994; 44: 75-80.

289. Houpt JB, McMillan R, Wein C, Paget-Dellio SD. Effect of glucosamine hydrochloride in the treatment of pain of osteoarthritis of the knee. J Rheumatol 1999; 26: 2423-30.

290 Shankland WE. The effects of glucosamine and chondroitin sulphate on osteoarthritis of the TMJ: A preliminary report of 50 patients. J Craniomand Pract 1998; 16: 230-235.

291. Hardingham TE, Fosang AJ. Proteoglycans: many forms and many functions. FASEB J 1992; 6:861-70.

292. Anderson MA. Oral chondroprotective agents. Part I. Common compounds. Compendium 1999; 21: 601-09.

293. Kelly GS. The role of glucosamine sulphate and chondroitin sulphate in the treatment of degenerative joint disease. Altern Med Rev. 1998; 3: 27-39.

294. Dettmer N. The therapeutic effect of glycosaminoglycan polysulfate (Arteparon) in arthroses depending on the mode of administration (intraarticular or intramuscular). Zeit fur Rheum 1979; 38:163-81.

295. Rovetta G, Monteforte P. Galactosaminoglycuronglycan sulfate in erosive osteoarthritis of the hands: early diagnosis, early treatment. Int J Tiss Reac 1996 18:43-6.

296. Rovetta G. Galactosaminoglycuronoglycan sulfate (matrix) in therapy of tibiofibular osteoarthritis of the knee. Drugs Exp Clin Res 1991; 17:53-7.

297. Bourgeois P, Chales G, Dehais J, Delcambre B, Kuntz JL, Rozenberg S. Efficacy and tolerability of chondroitin sulfate 1200 mg/day vs chondroitin sulfate 3 x 400 mg/day vs placebo. Osteoarthritis Cart 1998 6 (Suppl A):25-30.

298. Bucsi L, Poor G. Efficacy and tolerability of oral chondroitin sulfate as a symptomatic slow-acting drug for osteoarthritis (SYSADOA) in the treatment of knee osteoarthritis. Osteoarthritis Cart 1998; 6 (Suppl A):31-6.

299. Uebelhart D, Thonar EJ, Delmas PD, Chantraine A, Vignon E. Effects of oral chondroitin sulfate on the progression of knee osteoarthritis: a pilot study. Osteoarthritis Cart 1998; 6 (Suppl A):39-46.

300. Verbruggen G. Goemaere S. Veys EM. Chondroitin sulfate: S/DMOAD (structure/disease modifying anti-osteoarthritis drug) in the treatment of finger joint OA. Osteoarthritis Cart 1998 6 (Suppl A):37-8.

301. Morreale P, Manopulo R, Galati M, Boccanera L, Saponati G, Bocchi L. Comparison of the antiinflammatory efficacy of chondroitin sulfate and diclofenac sodium in patients with knee osteoarthritis. J Rheumat 1996; 23:1385-91.

302. Baici A, Horler D, Moser B, Hofer HO, Fehr K, Wagenhauser FJ. Analysis of glycosaminoglycans in human serum after oral administration of chondroitin sulfate [see comments]. Rheumat Int 1992; 12:81-8.

303. Conte A, Volpi N, Palmieri L, Bahous I, Ronca G. Biochemical and pharmacokinetic aspects of oral treatment with chondroitin sulfate. Arzneim-Forsch 1995; 45:918-25.

CHAPTER 2

EVALUATION OF GLUCOSAMINE SULPHATE AND IBUPROFEN FOR TREATMENT OF TEMPOROMANDIBULAR OSTEOARTHRITIS: A RANDOMIZED DOUBLE BLIND CONTROLLED 3-MONTH CLINICAL TRIAL

2.1 INTRODUCTION

Osteoarthritis is a degenerative disease of synovial joints characterized by a progressive loss of normal structure and function of articular cartilage. Osteoarthritis brings discomfort and disability to millions of North Americans each year and the costs involved in treating OA are expected to reach 1% of the United States gross national product in year 2000[1]. Its pathogenesis, although having been correlated to joint use, age and 'wear and tear', remains uncertain.

The TMJ is not immune in development of OA (also referred to as DJD) and a recent review reports approximately 8% to 12% of patients seeking treatment at temporomandibular dysfunction clinics receive a diagnosis of DJD[2]. Once the diagnosis is made, and if pain is an issue, the clinician generally places the patient on a soft diet, advises jaw functioning within a pain-free range, and prescribes an NSAID[2]. Nonsteroidal anti-inflammatories such as ibuprofen, have traditionally been the medicines of first choice[3,4].

Nonsteroidal anti-inflammatory drugs have a well-documented record of relieving pain and reducing inflammation. Unfortunately many of these medications are known to cause multiple side effects, notably upper GI damage[5]. It has been reported that 14.6% to 43.9% of OA patients treated with traditional NSAID's develop gastric ulcers after 6

months of therapy[6]. Epidemiological and clinical studies report that the cost of NSAID treatment should be multiplied by a coefficient range of 1.36 to 3 when the cost of treating the induced GI damage is also taken into account[7].

There is now a growing body of evidence that many of the more traditional NSAID's exacerbate the loss of the articular cartilage necessary for joint health by inhibiting proteoglycan synthesis at the level of the chondrocyte[8-12]. This problem has prompted research into medicinal agents that have a cartilage sparing, regenerative capacities and pain relieving effects.

Glucosamine is a naturally occurring aminomonosaccaride in the human body, biosynthesized from glucose and used to form GAG's, a constituent of proteoglycans which is an important component of the ECM of articular cartilage[13]. It's potential as a therapeutic agent for OA was first reported in 1969[14]. Investigations in the early 1980's found those patients with OA of the knee when administered glucosamine compared to placebo reported gradual and progressive reduction of articular pain and tenderness and improvement in the range of motion[15-18]. Oral administration of GS has also been reported to not irritate the GI tract[19] and may stimulate the production of protective gastric mucoproteins[20]. Several studies have also reported that therapeutic benefits of GS were maintained for weeks after therapy was discontinued[21,22].

Glucosamine sulphate is regarded as a food supplement and is available in health food and drug stores. It's potential as an adjunctive medicine for OA is gaining growing acceptance, supported by tissue, animal and human studies (please see discussion). Like other joints of the body, traditional pharmacological methods for treating patients with OA of the TMJ have largely depended on NSAID's[23]. "Natural medicines" like GS may provide symptom relief for this patient population without the inherent side effects of many traditional NSAID's. To date there are no published clinical trials to assess the efficacy of GS in treatment of those patients with DJD of the TMJ

with pain. The articular surface of the TMJ is composed of a dense fibrous connective tissue (also referred to as fibrocartilage) and direct comparison to other synovial joints with hyaline cartilaginous articular surfaces may not be appropriate. It was therefore the purpose of this study to investigate the potential of GS for treating patients diagnosed with TMJ DJD with pain.

2.2 MATERIALS AND METHODS

Forty-five individuals from a total of 176 interviewed (156 females, 20 males) over a 16 month period were diagnosed with DJD of one or both TM joints and deemed eligible to participate in this study. Participants were either patients of our Orofacial Pain Clinic, at the University of Alberta, or recruited via mail out to Edmonton and surrounding area dentists or through local newspaper advertisement. Of the females not recruited (116), 45 did not show radiographic evidence of OA, 30 had inadequate pain levels, 10 reported allergy to NSAID's and 31 did not proceed for radiographic assessment. Of the male's not recruited (15), 10 did not show radiographic evidence of OA, 3 had inadequate pain levels, and 2 reported allergy to NSAID's. In addition to exclusion criteria set for this study (Table 2.1), patients met diagnostic criteria for DJD established by the American Board of Orofacial Pain[24] including radiographic evidence of DJD confirmed by polycycloidal axially corrected tomographic radiographs (Tomax – Incubation Industries Inc. 429 Easton Rd. Warrington, Pennsylvania, USA, 18976) and a minimum mean pretreatment TMJ pain on function (pain on chewing, yawning, laughing and talking) visual analogue scale (VAS) score of 3 out of 10. Three was used since it is considered the lower boundary in establishing a moderate pain level for a 1 to 10 VAS scale[25] and moderate pain levels are required before administration of analgesic agents to ensure adequate sensitivity of treatment effect[26].

This study was conducted in a double blind manner. Neither the patients nor the investigators knew which of the two medications was administered until the end of the study and medications were prepared and coded as identical clear capsules by a local pharmacist from batches that came with certificate of analysis of ingredients to ensure uniformity throughout (Appendix I). Jamieson™ (Windsor, Ontario, Canada) and Apotex Co. (Toronto, Ontario, Canada) kindly donated GS and ibuprofen respectively. There was no drug crossover since carry over effects have been reported for GS[21,22]. Patients were block randomized into one of the two treatment groups, GS (500mg) and ibuprofen (400mg). The advantage of block randomization is that it ensures that the number of participants is equally distributed among the treatment groups over the coarse of the study. There was a one-week pretreatment washout period for all patients to eliminate the potential effects of previously used NSAIDs and/or analgesics. Participants were instructed to take the medication q8h with food and allowed only acetaminophen tablets (500mg), 1-2 q4-6h prn (maximum 4000 mg per day) for breakthrough pain. Patients returned every 30 days to count acetaminophen used and dispensed the next 30 days of study medication.

The primary clinical outcome in this trial was a reduction in joint pain with function (chewing, yawning, talking, laughing) measured using a modified visual analogue scale, the coloured analogue scale (CAS) developed by McGrath *et al*[27]. The CAS, used in pediatric pain, is a modification of the visual analogue scale, a valid and reliable pain measurement tool [28-30].

Secondary outcomes measured were: 1) Pain free and voluntary maximum interincisal opening, measured with a 100 mm ruler. 2) Brief Pain Inventory questionnaire, a valid and reliable questionnaire that measures pain intensity and impact (interference) on quality of life[31]. Intensity (worst and least pain in the last week, average pain, pain right now) recorded on numerical scales running from 0 (no pain) to 10 ("pain

as bad as you can imagine"). The impact of the pain was recorded in terms of how much it interferes with general activity, mood, walking ability, normal work, relations with others, enjoyment of life, sleep, recorded on a numerical scale running from 0 (does not interfere) to 10 (completely interferes). Permission to use this questionnaire was given by Dr. Charles S. Cleeland (developer) on March 9, 1998. 3) Extraoral masticatory muscle tenderness (7 equivalent sites bilaterally (total of 14) was assessed using a pressure threshold meter (algometer) with palpation sites located according to Kim *et al* 1996[32]. Muscles assessed were the anterior, middle and posterior temporalis, anterior, inferior and deep masseter and medial pterygoid. The algometer is used to obtain the pressure pain threshold (PPT), the minimum pressure (force) inducing discomfort or pain. Validity[33-36] and reliability[33,34,37,38] of this measure has been reported. The pressure algometer used was the Baseline® push/pull dynamometer (GNR Orthopaedic and Rehabilitation Products, Ocala Florida), a hand held force gauge fitted with a soft rubber disk with a surface area of $1cm^2$. The gauge is calibrated in kg/cm^2, with a range to 5 kg and 50 g divisions. The recording procedure has been previously described[39] and consists of placing the tip of the gauge perpendicular to the site of interest, increasing the pressure at a rate of 1kg/second. The pressure was stopped and the gauge removed for reading when the patient says "yes" indicating her/his pressure threshold. Pressure pain threshold values (in kilopascals) for masticatory muscles of specific interest in our study have been previously reported in females and males without history of headache, facial, or neck pain[40] (Appendix II). Our study used the mean minus one standard deviation values reported by Chung *et al*[40] and converted them to kg/cm^2 units according to the formula kg = 0.0102 X kPa/kPa. Readings obtained in our study that were equal to or below these "normal" PPT values were noted as positive responses and those that were equal to or higher than the normal values were noted as negative responses. A value of 1 is assigned for each positive response and 0 for each negative

response. In this way a palpation index similar to that described by Fricton *et al*[41] for masticatory muscles was used to access muscle tenderness. The index used in this current study is the sum of the positive responses out of the total number of sites (in this case 14).

Appendix III presents the intra-rater reliability of the three measurement tools used (100mm ruler, pressure algometer, CAS) for all variables measured.

Statistical analysis involved paired t-tests for within group analysis and independent sample t-test for between group analysis. Statistical significance was set at alpha 0.05. Data were analyzed using SPPS software, version 9.0 for windows.

The University of Alberta Ethics Committee approved this study April of 1998.

2.3 RESULTS

One hundred and seventy-six patients were interviewed for this study from August 1st , 1998 to November 1st , 1999. Of the 45 patients that qualified (mean age 37.5 years; 40 females, 5males), 39 (87%) completed the study. The most common reason for exclusion was the lack of radiographic evidence of DJD (31%). Four (9%) patients taking ibuprofen and 2 (4%) patients taking GS discontinued the study due to side effects. Three of the four dropouts in the ibuprofen group discontinued due to stomach upset (dropout at day 7 for two of these, day 57 for the other), the other due to inadequate pain control (dropout at day 64). One dropout in the GS group was due to dizziness (dropout day 43) the other due to stomach upset (dropout day 34).

There were no significant differences between treatment groups in terms of demographic characteristics or measured variables at the start of the study (Appendix IV).

When all patient data was analyzed, there were significant improvements from baseline (day 0) to day 90 for all variables measured (except acetaminophen used) for both treatments (Table 2.2).

Six (29%) and 7 (39%) of patients taking either GS or ibuprofen respectively did not respond when clinical significance was set as a 20% improvement in TMJ pain with function. Between-group analysis of differences for patients showing at least 20% reduction in functional pain revealed that participants taking GS improved significantly greater in terms of functional pain evaluation and overall pain interference than those taking ibuprofen (Table 2.3). In addition, patients that had taken GS used significantly less acetaminophen than the ibuprofen group from day 90 to 120.

When clinical significance for functional pain improvement is set at 20% and above no significant differences are found between the two treatment groups other than when set at greater than 80% (Table 2.4).

2.4 DISCUSSION

Over the past five years public interest in GS for treatment of OA has increased due in part by two non scientific publications, *The Arthritis Cure*[42] and *Maximizing the Arthritis Cure*[43]. The lack of interest in GS by researchers and pharmaceutical companies in general has been attributed by some to the fact that glucosamine is a natural product that cannot be patented[44].

The potential of glucosamine as a therapeutic agent for OA was first reported in 1969 by a German physician[14]. Approximately a decade later other German investigators[45-47] reported decreases in pain often accompanied by increased mobility when patients received a 400 mg solution of GS once daily administered either intravenously, intramuscularly or intraarticularly. These results should not be considered definitive since they were uncontrolled studies. Numerous controlled, double-blind

investigations evaluating glucosamine (oral, intramuscular or intravenous administration) versus placebo in patients diagnosed with OA of the knee were carried out in the early 1980's[15-18]. All studies reported gradual and progressive reduction of articular pain, joint tenderness and swelling, and improvement in the range of motion. A double-blind eight week study involving 40 patients with OA of the knee, found that GS 500 mg tid was as effective as Ibuprofen 400 mg tid in relieving pain after the first two weeks and by the end of the trial was more effective[48]. Although these studies reported improvement in symptoms when patients with OA of the knee were administered glucosamine, there were limitations in study design that included using hospitalized patients undergoing active physiotherapy, blinding placebo injections and short study times. These earlier studies have been critically reviewed[49-51].

A number of articles on GS have been published within the last decade. Muller-Fassbender and colleagues[52] in a double blind 4-week trial, randomized 200 patients with OA of the knee. These researchers found oral GS (500 mg tid.) just as effective as ibuprofen (400 mg tid.) from the second week of treatment and no difference was found between groups with respect to the magnitude of response. Adverse events were found to occur in 35% of the ibuprofen group, but in only 6% of the GS group, with fewer drop-outs in the latter. Qiu *et al*[53] in a similar 4 week trial of 178 Chinese patients found both GS (1500mg daily dose) and ibuprofen (1200mg daily dose) significantly reduced the symptoms of knee OA with a trend toward GS to be more effective. The GS group reported fewer adverse reactions (6%) compared to ibuprofen (16%) group and there were no dropouts in the GS group as compared to 10% in the ibuprofen group. Noack and colleagues[54], in a 4 week placebo controlled study of 252 patients with OA of the knee reported patients that had taken 1500 mg/day of GS orally showed significant improvement in the Lequesne index compared to the placebo group. Reichelt *et al.*[21] compared treatment of 400 mg of GS intramuscularly twice per week for 6 weeks with

placebo injections administered on the same schedule in 155 patients with OA of the knee. Fifty-five percent of patients who received GS and 33% of those given placebo responded as judged by the Lequesne index.

In the most recent study published, patients with OA of the knee in an 8 week double blind study were given either glucosamine hydrochloride (n=41), 500mg tid, or placebo (n=48)[55]. No statistically significant difference was found between groups in the primary endpoint measured (Western Ontario and McMaster University Osteoarthritis Index (WOMAC) pain score) between Week O and Week 8. However, significant differences from Week 5 to Week 8 in secondary endpoints (daily diary and knee examination) were found that suggested that glucosamine hydrochloride benefits some patients with knee OA.

There has been only one published report of GS for patients with TMJ OA[56]. The primary outcome of this study of 50 patients was a reduction in joint noise. All patients received glucosamine hydrochloride (1600 mg bid), 1000 mg of calcium ascorbate (1000 mg bid) and a mixture of chondroitin sulphate-4 and chondroitin sulphate-6 (1200 mg bid). Eighty percent of patients after an undefined period of time reported a reduction in joint noise. Unfortunately, this study does not indicate treatment time (other than patients were re-evaluated every 2-3 weeks) and cannot distinguish which of the co-administered supplements influenced outcome the most. In addition, the influence of either occlusal splint therapy introduced during the study for "many" patients or ibuprofen and aspirin permitted (counts not reported) when joint pain and/or swelling interfered with daily routines and activities, on the primary outcome in unclear. This study was neither randomized nor blinded. Reducing joint noise as a primary treatment outcome for TMD patients is questionable and the value of this study is uncertain.

Our study was designed to establish whether the food supplement GS, reported to help the symptoms of knee OA, will improve symptoms in patients diagnosed with OA

of the TMJ when compared to a more traditionally used pharmacological agent for OA. An overall $\geq$ 20% decrease in TMJ pain with functioning (chewing, yawning, talking, laughing) was our primary clinical outcome since a decrease in pain on function is often a primary reason why patients present for treatment at our clinic. Important secondary issues that often arise due to TMJ OA are decreases in pain free and voluntary mouth opening and masticatory muscle tenderness. Patients also report symptoms that fluctuate in severity over time and interfere with their daily functioning. All these secondary issues were addressed in this study.

Results from this study found that GS decreased TMJ pain on function, increased pain free and voluntary mouth opening and decreased the severity and interference the pain has on daily functions. Similar results were seen for the ibuprofen treatment group, but improvement in functional pain was significantly less than the GS treatment group and patients in the ibuprofen group found no significant improvement from the start of the study in terms of how the pain interfered with daily activities.

Both treatment groups used approximately equivalent amounts of acetaminophen for breakthrough pain throughout the study, but once study medication was discontinued the ibuprofen treatment group needed significantly more acetaminophen over the ensuing 30 days. This may be attributable to ibuprofen's ability to only help symptoms while a therapeutic dose is maintained, and a carryover effect for GS that has been previously reported[21,22].

Important questions clinicians are often asked by their patients are "how much will this medication help my joint pain and are there any side effects". Results form our study indicate that approximately 50% of patients that are prescribed GS will achieve atleast a 50% reduction in their joint pain on function and 70% of patients at least a 39% reduction of their pain. Side effects and dropouts in this study are comparable to past

literature comparing GS and ibuprofen. In general one can expect few side effects when taking GS and will mainly consist of minor GI problems.

Osteoarthritis of the TMJ is a prevalent and serious health care issue. Eight to 12% of patients that present to a TMD and Orofacial pain clinic receive a diagnosis of this disease[2]. These patients tend to be young females in their second and third decades of life[57-59], times we consider the most fruitful and productive. In addition, signs and symptoms of TMJ degenerative disease can occur in early childhood[60]. Professionals treating OA of the TMJ face similar dilemmas as those treating OA of other joints in terms of what's best to prescribe when pharmacotherapy is necessary. For the most part decisions have been based on knowledge acquired from research on joints other than the TMJ. Can we assume that "knowledge of OA in other synovial joints is appropriate to apply to the TMJ"[61]?

Fundamental differences exist between the TMJ and other synovial joints. A major difference is that the articular surface of the TM joint (mandibular condyle and the temporal fossa) is not cartilage but a dense fibrous connective tissue (also referred to as fibrocartilage) and consists primarily of type I collagen[62] rather than type II seen in hyaline cartilage[63]. Water constitutes over 65-70% of the total weight of hyaline cartilage[63] and cited as smaller in TMJ fibrocartilage[61]. Chondrocytes distributed throughout the extracellular matrix accounting for approximately 2%-3% of the total tissue volume in hyaline cartilage[63] but only 0.01%-0.1% of the total tissue volume in TMJ articular fibrocartilage[64]. The articular tissue of the TM joint consists mainly of fibroblasts not chondrocytes as in hyaline cartilage[62]. In hyaline cartilage, the glycosaminoglycan chains of proteoglycans consist of 90% chondroitin 4 and 6-sulphate and keratin sulphate[63]. Temporomandibular joint fibrocartilage, dermatan sulphate content has been cited as being higher, whereas keratan sulphate content is much smaller[61].

Our results add to a growing body of literature advocating the use of GS in OA but also to the controversy that exists with this food supplement. As of 1997, The Arthritis Foundation does not recommend the use of glucosamine as a treatment of OA[65]. GS has been termed a 'chondroprotective agent'[66,67], that has been defined as 'a substance able of increasing chondrocyte anabolic activity, while simultaneously suppressing the degradative action of mediators (cytokines, prostaglandins, proteinases) on cartilage'[66]. This term, however, has been considered misleading and inappropriate when applied to OA since OA is a process of the entire joint not only the articular cartilage[68]. The term disease modifying OA drug (DMOAD) (also called structure modifying drugs for OA) has been used to describe 'an agent that arrests or retards the progression of OA and/or enhances normal reparative processes in the diseased joint'[68]. To date, there have been no agents proven to have structure modifying properties in humans[69] and it is not known if GS has DMOAD activity. At this point it may be more appropriate to describe GS as a 'symptom modifying agent' since improvement in joint pain is reported for most clinical trials to date and improvement in joint pain is recommended as the primary outcome measure for symptom modifying agents[69].

An *in vitro* study in the 1950's showed that glucosamine stimulated the uptake of $^{35}SO_4^-$, a marker of glycosaminoglycan synthesis by cartilage[70]. Glucosamine sulphate has been found to significantly increase *in vitro* secretion of glycosaminoglycans by fibroblast cultures[71]. Other research in the 1970's and early 1980's have also demonstrated that exogenous glucosamine increased the synthesis of glycosaminoglycans in cartilage cultures[72,73]. More recently, using chondrocytes isolated from and cultured from human osteoarthritic femoral heads has found that GS induces a significant and dose dependent increase of proteoglycan synthesis but did not effect DNA synthesis or collagen type II or PGE_2 production by chondrocytes[74].

Using animal models of inflammation oral glucosamine was reported to protect rats from inflammation caused by several non-specific foreign agents (dextran, formalin and acetic acid) but does not exert activity against specific mediators of inflammation (histamine, serotonin or bradykinin)[19,75]. Glucosamine sulphate has no analgesic activity, is ineffective against proteolytic enzymes of inflamed tissues and against the biosynthesis of prostaglandin's elicited from arachidonic acid or histamine[19,75]. Glucosamine sulphate reduces superoxide radicals generated by macrophages, inhibits lysosomal enzymes and its effects are prostaglandin independent[19,75]. Glucosamines effects have been described as a prostaglandin independent, cyclooxygenase independent "antireactive" activity[19,75].

The biochemical events to explain symptom relief in patients taking GS are not completely known but perhaps only partially explained by GS's ability to act as a substrate and stimulant of glycosaminoglycan production within articular cartilage. Has a focus on articular cartilage alone become a 'red herring'? Osteoarthritis is a disease process of the entire joint that includes the synovial membrane and subchondral bone and not just the aneural articular tissues. Traditional NSAIDs' decrease symptoms by inhibiting cyclooxygenase but can also interfere with cartilage metabolism. Glucosamine sulphate induces cartilage metabolism and its effects are cyclooxygenase independent. Can symptom relief be entirely explained by cartilage metabolism effects or are there secondary events such as inhibition of catabolic mechanisms of OA induced by pro-inflammatory cytokines such as interleukin-1 and tumor necrosis factor alpha that explain GS's effects? This may warrant further research and provide more insight as to GS's role in OA.

This study was designed to evaluate whether GS would help the symptoms of TMJ OA that many patients present with at our Orofacial Pain Clinic. Our results, although only the first for the TMJ, indicate that GS has at least the same potential as a

traditional medication prescribed for OA and temporomandibular disorders. It is too early, however, to make definitive conclusions on this food supplement since limitations were encountered and include; 1) Low statistical power that would be improved with a larger study population - a post hoc analysis of our results, with a power of 0.80, revealed that 82 patients would be more suitable for future comparative studies with GS. 2) Large variances in the results, perhaps attributable in part to psychological aspects known to influence the pain experience- difficult to measure clinically. 3) No placebo control - although our research did find that once patients taking GS discontinued this medication less acetaminophen was required for pain over an ensuing 30 days than the ibuprofen treatment group which is not indicative of a placebo response.

REFERENCES

1. Yelin E. The economics of osteoarthritis. In: Brandt M, Doherty M, Lohmander LS, editors. Osteoarthritis. Oxford: Oxford Medical Publications; 1998: 23-30.
2. Kamelchuk LS, Major PW. Degenerative disease of the temporomandibular joint. J Orofacial Pain 1995; 9: 168-180.
3. Gangarosa LP, Mahan PE, Ciarlone AE. Pharmacologic management of temporomandibular joint disorders and chronic head and neck pain. J Cranio Practice 1991; 9: 328-38.
4. Gray RJM, Davies SJ, Quayle AA. A clinical approach to temporomandibular disorders. A clinical approach to treatment. Br Dent J 1994; 177: 101-06.
5. Agrawal NM. Anti-inflammatories and gastroduodenal damage: Therapeutic options. Eur J Rheum Inflam 1993; 13: 17-24.
6. Geis GS, Stead H, Wallemark CB, Nicholson PA. Prevalence of mucosal lesions in the stomach and duodenum due to chronic use of NSAIDs in patients with rheumatoid arthritis or osteoarthritis, and interm report on prevention by misoprostol of diclofenac associated lesions. J Rheumatol 1991;18 Suppl 28: 11-14.
7. de Pouvourville G, Tasch RF. The economic consequences of NSAID-induced gastrointestinal damage. Eur J Rheum Inflam 1993; 13: 33-40.
8. Simon LS. Biologic effects of nonsteroidal anti-inflammatory drugs. Curr Opin Rheumatol 1997; 9: 178-82.
9. Brandt KD. Should nonsteroidal anti-inflammatory drugs be used to treat osteoarthritis ? Rheum Dis Clin North Am 1993; 19: 29-44.
10. Brandt KD. Should osteoarthritis be treated with nonsteroidal anti-inflammatory drugs? Rheum Clin Dis North Am 1993; 19(3): 697-712.

11. Huskisson EC, Berry H, Gishen P, Jubb RW, Whitehead J. Effects of anti-inflammatory drugs on the progression of osteoarthritis of the knee. J Rheumatol 1995; 22: 1941-46.

12. Sheild MJ. Anti-inflammatory drugs and their effects on cartilage synthesis and renal function. Eur J Rheum Inflamm 1993; 13(1): 7-16.

13. Lehninger AL. Biochemistry. New York: Worth Publisher; 1975.

14. Bohne W. Glukosamine in der konservativen arthrosebehanlung. Med Welt 1969; 30: 1668-71.

15. Crolle G, D'Este E. Glucosamine sulphate for the management of arthrosis: a controlled clinical investigation. Curr Med Res Opin 1980; 7: 104-09.

16. Drovanti A, Bignamini AA, Rovati AL. Therapeutic activity or oral glucosamine sulphate in osteoarthrosis: a placebo-controlled double-blind investigation 1980; Clin Ther 3: 260-72.

17. D'Ambrosio E, Casa B, Bompani R. Glucosamine sulphate: a controlled clinical investigation in arthrosis. Pharmatherapeutica 1981; 2: 504-08.

18. Pujalte JM, Llavore EP, Ylescupidez FR. Double-blind clinical evaluation of oral glucosamine sulphate in the basic treatment of osteoarthrosis. Curr Med Res Opin 1980; 7: 110-14.

19. Setnikar I, Pacini MA, Revel L. Antiarthritic effects of glucosamine sulphate studied in animal models. Arzneim Forsch 1991; 41: 542-45.

20. Moriga M, Aona M, Murakami M, Uchino H. The activity of N-acetylglucosamine kinase in rat gastric mucosa. Gastroenterol Japonica 1980; 15: 7-13.

21. Reichelt A, Forster KK, Fischer M, Rovati LC, Setnikar I. Efficacy and safety of intramuscular glucosamine sulphate in osteoarthritis of the knee. Arzneim Forsch 1994; 44: 75-80.

22. Tapadinhas MJ, Rivera IC, Bignamini AA. Oral glucosamine sulphate in the management of arthrosis: report on a multi-centre open investigation in Portugal. Pharmatherapeutica 1982; 3: 157-68.

23. Pertes RA, Cohen HV. Guidelines for clinical management of temporomandibular disorders: Part 2. Compend Contin Educ Dent 1992; 13: 400-13.

24. American Academy of Orofacial Pain In: Okeson JP editor. Orofacial Pain: guidelines for assessment, classification, and management. Carol Stream, Illinois: Quintessence; 1996: 135-37.

25. Collins SL, Moore A, Mcquay HJ. The visual analogue pain intensity scale: what is moderate pain in millimetres? Pain 1997; 72: 95-97.

26. Lasagna L. The psychophysics of clinical pain. Lancet 1962; 2: 572-75.

27. McGrath PA, Seifert CE, Speechley KN, Booth JC, Stitt L, Gibson MC. A new analogue scale for assessing children's pain: an initial validation study. Pain 1996; 64: 435-43.

28. Chapman RC, Syrjala KL. Measurement of pain. In: Bonica JJ, editors. The management of pain, 2nd ed. Philadelphia: Lea and Febiger; 1990: 580-94.

29. Jensen MP, Karoly P. Self-report scales and procedures for assessing pain in adults. In: Turk DC, Melzack R, editors. Handbook of pain assessment. New York: Guilford; 1992:135-151.

30. Wewers ME, Lowe NK. A critical review of visual analogue scales in the measurement of clinical phenomena. Res Nurs Health 1990; 13: 227-236.

31. Cleeland CS. The Brief Pain Inventory. In: McDowell I, Newell C, editors. Measuring Health, 2nd ed. New York: Oxford University Press; 1996: 352-57.

32. Kim HS, Chung SC, Kim YK, Lee SW. Pain-pressure threshold in the head and neck region of episodic tension-type headache patients. J Orofacial Pain 1995; 9: 357-64.

33. Jensen K. Quantification of tenderness by palpation and use of pressure algometers. In: Fricton JR, Awad E, editors. Advances in pain research and therapy, Vol. 17. New York: Raven Press; 1990: 165-81.

34. Ohrbach R, Gale EN. Pressure pain thresholds, clinical assessment, and differential diagnosis: reliability and validity in patients with myogenic pain. Pain 1989; 39: 157-69.

35. Reeves JL, Jaeger B, Graff-Radford SB. Reliability of the pressure algometer as a measure of myofascial trigger point sensitivity. Pain 1986; 24: 313-321.

36. Fischer AA. Pressure algometry over normal muscles. Standard values, validity and reproducibility of pressure threshold. Pain 1987; 30: 115-126.

37. McMillan AS, Blasberg B. Pain-pressure threshold in painful jaw muscles following trigger point injection. J Orofacial Pain 1994; 8: 384-90.

38. Reid KI, Gracely RH, Dubner RA. The influence of time, facial side, and location on pain pressure thresholds in chronic myogenous temporomandibular disorder. J Orofacial Pain 1994; 8: 258-65.

39. Fischer AA. Documentation of myofascial trigger points. Arch Phys Med Rehabil 1988; 69: 286-291.

40. Chung SC, Um BY, Kim HS. Evaluation of pressure pain threshold in head and neck muscles by electronic algometer: Intrarater and interrater reliability. J Craniomandib Pract 1992; 10: 28-34.

41. Fricton JR, Bromaghim C, Kroening RJ. Physical Evaluation: The need for a standardized exam In: Fricton JR, Kroening RJ, Hathaway KM, editors. TMJ and Craniofacial Pain: diagnosis and management: St Louis: Ishiyaku EuroAmerica;. 1988: 46-47.

42. Theodosakis J, Adderly B, Fox B. The Arthritis Cure. New York: St. Martin's Press. 1997.

43. Theodosakis J, Adderly B, Fox B. Maximizing the Arthritis Cure. New York: St. Martin's Press. 1998.

44. McCarty MF. The neglect of glucosamine as a treatment for osteoarthritis-a personal perspective. Medical Hypothesis 1994; 42: 323-27.

45. Eichler J, Noh E. Therapy of deforming arthrosis through the action upon the cartilaginous metabolism. Orthop Praxis 1970; 6: 225-29.

46. Dustmann HO, Puhl W. Die intraartikulare injektionstherapie der arthrose-klinische und tierexperimentelle untersuchungen. Orthop Praxis 1978; 14: 682-84.

47. Mund-Hoym WD. Die behandlung von Huft and Kniegelenkarthrosen. Z Allg Med 1980; 56: 2153-2159.

48. Vas AL. Double-blind clinical evaluation of the relative efficacy of ibuprofen and glucosamine sulphate in the management of osteoarthrosis of the knee in out-patients. Curr Med Opin 1980; 8: 145-149.

49. Barclay TS, Tsourounis C, McCart GM. Glucosamine. Ann Pharmacother 1998; 32: 574-79.

50. da Camara CC, and Dowless GV. Glucosamine sulphate for osteoarthritis. Ann Pharmacother 1998; 32: 580-87.

51. Deal CL and Moskowitz RW. Nutraceuticals as therapeutic agents in osteoarthritis: The role of glucosamine, chondroitin sulphate and collagen hydrolysate. Rheum Disease Clinics North Am 1999: 25: 379-395.

52. Muller-Fassbender H, Bach GL, Haase W, Rovati LC, Setnikar I. Glucosamine sulphate compared to ibuprofen in osteoarthritis of the knee. Osteoarthritis Cart 1994; 2: 61-69.

53. Qiu GX, Gao SN, Giacovelli G, Rovati L, Setnikar I. Efficacy and safety of glucosamine sulphate versus ibuprofen in patients with knee osteoarthritis. Arzneim-Forsch/Drug Res 1998; 48: 469-474.

54. Noack W, Fischer M, Forster KK, Rovati LC, Setnikar I. Glucosamine sulphate in osteoarthritis of the knee. Osteoarthritis Cart 1994; 2: 51-59.

55. Houpt JB, McMillan R, Wein C, Paget-Dellio SD. Effect of glucosamine hydrochloride in the treatment of pain of osteoarthritis of the knee. J Rheumatol 1999; 26: 2423-30.

56. Shankland WE. The effects of glucosamine and chondroitin sulphate on osteoarthritis of the TMJ: A preliminary report of 50 patients. J Craniomand Pract 1998; 16: 230-235.

57. Wiberg B, Wanman A. Signs of osteoarthrosis of the temporomandibular joints in young patients. A clinical and radiographic study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 86: 158-64.

58. Dijkgraaf LC, Liem RSB, de Bont LGM. Synovial membrane involvement in osteoarthritic temporomandibular joints. A light microscopic study. . Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997; 83: 373-86.

59. Bates RE, Gremillion HA, Stewart CM. Degenerative joint disease. Part I: Diagnosis and management considerations. J Cranimand Pract 1993; 11: 284-290.

60. Dibbets JMH, Weele van der Lt, Boering G. Craniofacial morphology and temporomandibular joint dysfunction in children. In: Carlson DS, McNamara JA, Ribbens KA editors. Development aspects of temporomandibular joint disorders monograph 16, craniofacial growth series. Centre for human growth and development Ann Arbor; The University of Michigan 1985: 151-182.

62. Dijkgraaf LC, de Bont LGM, Boering G, Liem RSB. Normal cartilage structure, biochemistry, and metabolism: A review of the literature. J Oral Maxillofac Surg 1995; 53: 924-29.

63. Cate ART. Gross and micro anatomy. In: Zarb GA, Carlsson GE, Sessle BJ, Mohl ND editors. Temporomandibular joint and masticatory muscle disorders. Copenhagen, Munksgaard, 2nd ed. 1994; 48-66.

64. Thonar EJ-MA, Masuda K, Manicourt DH, Kuettner KE. Structure and function of normal human adult articular cartilage. In: Reginster JY, Pelletier JP, Martel-Pelletier J, Henrotin Y, editors. Osteoarthritis: Clinical and experimental aspects. Berlin: Springer; 1999: 1-19.

65. de Bont LG. de Haan P. Boering G. Structure and growth of the cartilage of the temporomandibular joint. Nederlands Tijdschrift voor Tandheelkunde. 1985; 92:184-9.

65. Arthritis Today. Vol 11 (May/June). Atlanta: Arthritis Foundation, 1997.

66. Bassleer C, Henrotin Y, Franchimont P. In-vitro evaluation of drugs proposed as chondroprotective agents. Int J Tiss Reac 1992; 14: 231-41.

67. Anderson MA. Oral chondroprotective agents. Part I: Common compounds. Compendium 1999; 21: 601-09.

68. Altman RD, Howell DS. Disease-modifying osteoarthritis drugs. In: Brandt KD, Doherty M, Lohmander LS, editors. Osteoarthritis. Oxford: Oxford University Press; 1998: 417-428.

69. Altman R. Brandt K. Hochberg M. Markowitz R. Bellamy N. Bloch DA. Buckwalter J. Dougados M. Ehrlich G.Lequesne M. Lohmander S. Murphy WA Jr. Rosario-Jansen T. Schwartz B. Trippel S. Design and conduct of clinical trials in patients with osteoarthritis: Recommendations from a task force of the Osteoarthritic Research Society. Osteoarthritis &Cartilage. Vol 4(4) (pp 217-243), 1996.

70. Roden L. Effect of hexosamine on the synthesis of chondroitin sulphuric acid in vitro. Ark Kemi 1956; 10: 345-52.

71. Karzel K. Domenjoz R. Effects of hexosamine derivatives and uronic acid derivatives on glycosaminoglycane metabolism of fibroblast cultures. Pharmacology. 1971; 5: 337-45.

72. Vidal y Plana RR, Bizzarri D, Rovati AL. Articular cartilage pharmacology: I. In vitro studies on glucosamine and nonsteroidal antiinflammatory drugs. Pharmacol Res Comm 1978 10:557-69.

73. Vidal y Plana RR, Karzel K. Glucosamine: its importance for the metabolism of articular cartilage. 2. Studies on articular cartilage. Fortschritte der Medizin 1980; 98: 801-6.

74. Bassleer C, Rovati L, Franchimont P. Stimulation of proteoglycan production by glucosamine sulphate in chondrocytes isolated from human osteoarthritic articular cartilage in vitro. Osteoarthritis Cart 1998; 6: 427-34.

75. Setnikar I, Cereda M, Pacini MA, Revel L. Antireactive properties of glucosamine sulphate. Arzneim Forsch 1991; 41: 157-161.

TABLE 2.1 .

EXCLUSION CRITERIA.

Age < 18 years
Baseline pain intensity < 3/10 VAS
DJD as a result of acute trauma, previous infection or general joint/muscle disease
(e.g. rheumatoid arthritis
History of congestive heart failure, renal disease or hepatic disease
History of peptic ulceration or GI bleeding
Coagulation disorders
Pregnant or nursing mothers
History of hypersensitivity to NSAID's
Active dental disease, periodontal disease, oral infection or pathology,
Using antidepressant or anxiolytic medication
Using an occlusal splint for < 3months
Unwilling to give informed consent
Unwilling to take oral medication
Unwilling to undergo a one week washout
Unable to understand English

TABLE 2.2.

WITHIN TREATMENT GROUP COMPARISON DAY 90 TO BASELINE.

Variable	*Mean difference (SD) Glucosamine sulphate n=21	Coefficient of variation (SD/mean) Glucosamine sulphate n=21	p	*Mean difference (SD) Ibuprofen n=18	Coefficient of variation (SD/mean) Ibuprofen n=18	p
1) Functional pain evaluation (VAS)	-10.50(10.79)	1.03	.00	-5.93(5.83)	0.98	.00
2) Pain free mouth opening (mm)	10.14(11.09)	1.09	.00	9.39(7.41)	0.88	.00
3) Voluntary mouth opening (mm)	7.14(7.48)	1.05	.00	4.06(5.37)	1.32	.00
4) BPI questionnaire (VAS)						
i) Pain intensity	-10.00(8.92)	0.89	.00	-7.31(5.91)	0.81	.00
ii) Pain interference (VAS)	-15.07(13.68)	0.91	.00	-8.33(11.68)	1.40	.02
5) Extra oral masticatory muscle pain (positive on 14 sites)	-3.95(3.89)	0.99	.00	-4.33(4.54)	1.05	.01
6) Acetaminophen day 30 to 60	-2.95(10.04)	3.40	.19	1.78(11.39)	6.40	.52
7) Acetaminophen day 60 to 90	3.14(10.95)	3.48	.20	1.83(9.34)	5.10	.94
8) Acetaminophen day 90 to 120	-1.24(18.49)	14.91	.76	2.94(20.37)	6.92	.55

*Day 90 value minus beginning of study value

TABLE 2.3.

BETWEEN TREATMENT GROUP COMPARISON DAY 90 TO BASELINE WITH CLINICAL SIGNIFICANCE SET AT 20% IMPROVEMENT IN FUNCTIONAL PAIN.

Variable	*Mean difference (SD) Glucosamine sulphate n=15	Coefficient of variation (SD/mean) Glucosamine sulphate n=15	*Mean difference (SD) Ibuprofen n=11	Coefficient of variation (SD/mean) Ibuprofen n=11	p	Estimated power at observed values
1) Functional pain evaluation (VAS)	-15.19 (8.92)	0.59	-8.30(4.49)	0.54	0.02	0.62
2) Pain free mouth opening (mm)	12.93(11.62)	0.90	9.00(8.67)	0.96	0.35	0.15
3) Voluntary mouth opening (mm)	8.33(8.10)	0.97	6.46(5.94)	0.92	0.52	0.10
4) BPI questionnaire (VAS)						
i) Pain intensity	-13.13(8.33)	0.63	-8.23(4.89)	0.59	0.10	0.39
ii) Pain interference (VAS)	-19.50(12.32)	0.63	-8.64(14.27)	1.65	0.05	0.51
5) Extra oral masticatory muscle pain (positive on 14 sites)	-4.13(3.74)	0.91	-4.64(5.26)	1.13	0.78	0.06
6) Acetaminophen day 30 to 60	-3.53(10.68)	3.02	-1.82(11.44)	6.29	0.54	0.09
7) Acetaminophen day 60 to 90	1.80(9.68)	5.38	-2.18(7.68)	3.52	0.27	0.19
8) Acetaminophen day 90 to 120	-5.00(13.02)	2.60	8.55(10.59)	1.24	0.01	0.78
9) Total acetaminophen taken at 90	59.33(51.49)	0.87	42.27(69.35)	1.64	0.11	0.11

*Day 90 value minus beginning of study value

TABLE 2.4.

PERCENTAGE FUNCTIONAL PAIN IMPROVEMENT AT VARIABLE CLINICAL LEVELS OF SIGNIFICANCE

Set clinical level of significance(%)	Glucosamine sulphate n=21	Ibuprofen n=18	*p
Negative response	4(19%)	4(22%)	0.807
0 to19	17 (81%)	14 (78%)	0.807
20 to 39	15 (71%)	12 (67%)	0.749
40 to 59	10 (48%)	5 (28%)	0.191
60 to 79	6 (29%)	3 (17%)	0.367
80 to100	4 (19%)	0 (0%)	0.026

*Test for equality of proportions

CHAPTER 3

GENERAL DISCUSSION

3.1 INTRODUCTION

The main aim of this thesis was to investigate whether the food supplement GS would benefit patients diagnosed with TMJ DJD. The impetus for this research was the fact that many patients that present to our Orofacial Pain Clinic are young women who receive a diagnosis of TMJ DJD. Once the diagnosis is made and pain an issue, many are given home care instructions (e.g. soft diet, function within a pain free range of motion, etc.), referred for physiotherapy if deemed necessary, receive an occlusal splint appliance where appropriate and placed on anti-inflammatory medications. Ibuprofen has traditionally been the first line NSAID in treating TMD conditions[1-3]. Unfortunately, GI upset will force some patients to discontinue the medication leaving the clinician to decide what is appropriate for patients with persistent symptoms. In addition, traditional NSAID's such as ibuprofen may adversely affect the articular tissue of joints[4-8]. This adds to the challenge of using medications that provide symptom relief but with suitable side effect profiles.

Our interest in GS was to establish whether our patient population would receive any benefit from a food supplement which has recently received increased scientific and media attention. Our results although only the first for the TMJ finds GS may have at least the same potential as a traditionally used NSAID with fewer side effects. In addition, GS has an additional benefit of providing continued symptom relief after it is discontinued. Others[9,10] have also reported this carry-over effect, which may be

attributable to it's potential for being a substrate and stimulant of anabolic processes within articular tissues[11-15].

Osteoarthritis is a dynamic process involving all the joint structures with complex histological and biochemical processes. Symptom relief with traditional NSAID's is mediated via inhibition of COX pathways. Glucosamine sulphate's effects are COX independent and it is not considered a true anti-inflammatory[16,17]. It is evident that GS provides symptom relief but can this be entirely explained by effects within articular tissues alone? In other words is it possible for cartilage to "repair" and/or stabilize within a matter of months and translate into symptom relief? Might there be cartilage independent effects? Setnikar and colleagues[16,17] have reported that administered to rats in high oral doses (50-800 mg/kg), GS inhibited sponge and croton oil granulomas, carrageenin-induced edema, kaolin-induced and adjuvant arthritis, and peritonitis evoked by formalin. In mice, glucosamine inhibited acetic acid induced peritonitis. These authors also reported that glucosamine did not inhibit COX, nor the edema provoked by injection of the specific inflammatory mediators bradykinin, histamine or serotonin. Given that glucosamine did not inhibit the response to inflammatory mediators, Setnikar coined the term 'anti-reactive'- as opposed to 'anti-inflammatory' – to characterize it's activity. These authors found that glucosamine has no analgesic activity but was able to reduce the generation of superoxide radicals by macrophages and inhibit lysosomal enzymes. Further research is required to clarify the mechanism(s) of action of GS.

To date most of our knowledge and treatment of TMJ DJD has relied on knowledge gained from other synovial joints. In comparison very little is known about TMJ DJD but it has been assumed that since it is also a synovial joint it follows the same principals for OA as in other synovial joints[18]. Embryologically, however, the TMJ forms unlike other joints of the body and basic histological differences exist. Furthermore OA of the TMJ is mainly a disease of young women post puberty and premenopausal, not

postmenopausal as in other joints of the body. Certainly similarities may exist between OA of hyaline cartilaginous joints and the TMJ but generalizations are inappropriate.

The pathogenesis of TMJ OA remains a controversial issue. Some researchers suggest it is a result of mechanical overloading[19]. The role of disc displacement is also unclear, some believing that disc displacement precedes and predisposes to DJD[20] while others believe that disc displacement is a sign rather than a cause of DJD[21].

In the presence of disc displacement normal functional loads may result in adaptive tissue responses. At rest (closed mouth position) the condyle now rests on the highly innervated and vascularized retrodiscal tissues rather than the inferior surface of the disc. Because the disc is displaced, the opposing osseous articular surfaces (condyle and articular eminence) are now incongruous (since the disc usually fills the space between these two convex surfaces)[22]. With function these incongruous surfaces approximate with point localization's of load[22]. Normal reparative capacities may prevent damage and retrodiscal tissues remodel into a functional pseudodisc establishing a new equilibrium[23].

A source of lubrication necessary for normal joint mechanics is weeping lubrication, released from the disc and squeezed into the joint space between the loaded bony surfaces and the disc[24]. Altered disc position may result in decreased joint lubrication thereby increasing frictional resistance during loaded joint movements. In the presence of sustained load (e.g. parafunction) altered disc position along with decreased lubrication may predispose or exacerbate articular tissue failure. The diminution of adaptive repair leads to regressive remodelling and degeneration may follow[25].

Perhaps of greater value when discussing the etiopathogenesis of TMJ DJD is the metabolic and/or inflammatory tissue responses that occur in both fibrocartilage and synovial tissue. As previously discussed adaptive responses may follow disc displacement. This response represents a balance between repair and degradation of

ECM components and may be mediated in part by IGF-1, an important growth factor known to stimulate proteoglycan synthesis but also inhibit proteoglycan catabolism in chondrocytes[26]. In the presence of disc displacement sustained loading (clenching) and/or repetitive microtrauma (bruxing) or a single event trauma (macrotrauma) may result in damage to the articular tissues. Fibrocytes in reaction may now release increased amounts of proteolytic enzymes (MMP's) and catabolic cytokines resulting in breakdown of the ECM. This could correlate with increased levels of GAG components found in synovial fluid thought to reflect early signs of TMJ DJD[27-29]. The ECM degradation products in addition to other mediators produced by fibrocytes stimulate synovial macrophages and fibroblasts that in turn secrete a broad range of inflammatory mediators thereby inducing an observable synovitis[30-34]. An immune reaction may also be involved under conditions where the individual loses the tolerance against autoantigens from the articular tissue[35]. Cytokines (e.g. IL-1, TNFα) produced by the synovial membrane diffuse into the articular tissues through the synovial fluid and induce increased production of proteolytic enzymes and catabolic cytokines. The increased production of matrix degrading enzymes and cytokines relative to growth factors now results in the deterioration of articular tissue.

Loading and compression of the vascular synovial tissues in the presence of a displaced disc could exceed in magnitude the end capillary perfusion pressures of the tissue. When the intracapsular hydrostatic perfusion pressure exceeds the end-capillary perfusion pressure, blood flow is disrupted, resulting in tissue hypoxia[36]. Protracted episodes of hypoxia with long-term loading (clenching and bruxism) will affect the metabolic response of tissue. When tissue perfusion is reestablished blood now re-perfuses the subintimal tissues and the oxygen debt generated establishes a gradient for oxygen diffusion into joint tissues. The oxygen is now converted into damaging free

radicals. These oxygen radicals contribute to the persistence of chronic inflammation through this process termed hypoxic-reperfusion[37,38].

3.2 STUDY LIMITATIONS

There were a number of limitations and/or potential improvements that could have been made in this study. The first was patient sample size. Sample size estimation was based on previously published studies comparing ibuprofen and GS on patients with OA of the knee[39,40] - Appendix V. Sample size calculation indicated that 67 patients were needed per treatment group, however, only 45 in total were recruited. Recruiting for appropriate patients involved searching through 1000 active patient charts at our Orofacial Pain Clinic and 176 interviews of potential candidates acquired either through mailout to local dentists or local newspaper advertisement. The entire process demanded 16 months. Acquiring the ideal number of patients would have been beyond the time constraints of this research project.

The second limitation of this study was that there was no placebo control group. A three-armed study with placebo would have been ideal. However, because of difficulties in obtaining suitable study participants within a reasonable length of time a comparative study with ibuprofen was undertaken. Ibuprofen, 400 mg tid, has traditionally been recommended for pain relief of TMD symptoms[1-3] and most studies on GS compared to an NSAID have used ibuprofen 400mg tid[39-41]. Although anecdotal in nature many patients interviewed have said, "you're not using a placebo are you?" Patient compliance with placebo control would have been difficult.

The author of this thesis believes that DJD of the TMJ is a chronic inflammatory condition. An argument can be made then that ibuprofen used should have been 1800-2400 mg/day (anti-inflammatory dose) rather than 1200 mg /day (analgesic dose). The primary endpoint of this thesis, however, was a reduction in pain rather than

inflammation and this choice was made from the fact that almost all patients that present to our clinic are searching for pain relief. In addition, several papers have reported similar efficacy between acetaminophen and ibuprofen at both an analgesic (1200mg) or an anti-inflammatory (2400 mg) daily dosage when treating patients with OA of the knee[42]. The argument can then be made that if a reduction in joint pain is the primary outcome then only acetaminophen need be used for the comparison group. Many consider that the therapeutic effects of NSAID's in OA result primarily from their analgesic rather than anti-inflammatory properties[45,46]. Although the literature suggest that joint symptoms in many patients can be adequately controlled without NSAID's, other studies also suggest that some patients prefer NSAID's[44]. For these patients it is important to minimize the risk associated with NSAID therapy by minimizing the dose taken per day[44]. For these reasons an analgesic dose of ibuprofen was chosen.

3.3 CLINICAL IMPLICATIONS

The results of this study indicate that GS may be useful for patients diagnosed with TMJ DJD. This food supplement appears to ease symptoms of OA with few side effects. In combination with other forms of therapy (occlusal splint appliance, physiotherapy etc.) it may complement more recently developed pharmacotherapeutic strategies for treating the symptoms of OA. In particular are NSAID's that are COX-2 specific now available in Canada and include Celebrex® (200-400 mg bid) and Vioxx® (12.5-50 mg qd). These NSAID's are purported to be effective against the mediators of inflammation but with no specific effects on the GIT. At this time there is no reason (other than cost) that patients with TMJ DJD cannot be prescribed either of these two NSAID's in addition to recommending the use of GS – 500mg 3 times/day. If cost is an issue, NSAID's with high but incomplete COX-2 specificity such as Relafen® (Nabumetone) are good alternatives. Acetaminophen can be recommended for breakthrough pain.

3.4 DIRECTIONS FOR FUTURE STUDIES

At this point in time most, if not all, medications prescribed for OA are useful for symptomatic management of this disease. As expressed many times throughout this thesis there can be side effects associated with many of the traditional NSAID's. Hence the dawn of the COX-2 specific NSAID's. These newer agents may prove (as did the traditional NSAID's) useful for alleviating pain, swelling and stiffness but with reduced side effect profiles. But are they useful for slowing, stopping or even reversing the structural changes of the disease? Research over last two decades or so has focussed on medicinal agents that have disease (or structure) modifying abilities. These agents have been termed chondroprotective agents by some[47,48] and more recently disease modifying OA drugs (DMOAD's)[49]. A DMOAD's has been described as an agent that 'arrests or retards the progression of OA and/or enhances normal reparative processes in the diseased joint'[49]. Table 3.1 shows a variety of pharmacologic agents that have been investigated for potential DMOAD effects. To date, none have been identified as an effective DMOAD in humans. I believe that further research is required to assess the potential of these agents for our patient population.

TABLE 3.1.

POTENTIAL DISEASE-MODIFYING OSTEOARTHRITIC DRUGS[49]

- Sulfated glycosaminoglycans
  - Glycosaminoglycan-peptide (GAG-peptide)
  - Glycosaminoglycan polysulfuric acid (GAGPS)
  - Pentosan polysulphate
  - Chondroitin sulphate
  - Glucosamine sulphate
- Non-sulfated glycosaminoglycans
  - Hyaluronic acid
- Agents acting on bone
  - Bisphosphonates
    - Etidronate
  - Calcitonin
- Anti-inflammatory agents
  - Nonsteroidal anti-inflammatory drugs
    - Piroxicam
    - Tenidap
    - Tiaprofenic acid
  - Glucocorticoids
    - Oral
    - Intra-articular
  - Anthroquinones
    - Diacerhein
  - Lipids
- Enzyme inhibitors
  - Tetracycline's
    - Doxycyline
    - Chemically-modified tetracycline's
  - Specific stromelysin inhibitors
  - Specific collagenase inhibitors
- Cytokines/growth factors
  - Growth hormone
  - Insulin-like growth factor-1
  - Transforming growth factor-β
  - Interleukin-1 receptor antagonist

3.5 REFERENCES

1. Gangarosa LP, Mahan PE, Ciarlone AE. Pharmacologic management of temporomandibular joint disorders and chronic head and neck pain. J Cranio Practice 1991; 9: 328-38.
2. Gray RJM, Davies SJ, Quayle AA. A clinical approach to temporomandibular disorders. A clinical approach to treatment. Br Dent J 1994; 177: 101-06.
3. Pertes RA, Cohen HV. Guidelines for clinical management of temporomandibular disorders: Part 2. Compend Contin Educ Dent 1992; 13: 400-13.
4. Simon LS. Biologic effects of nonsteroidal anti-inflammatory drugs. Curr Opin Rheumatol 1997; 9: 178-82.
5. Brandt KD. Should nonsteroidal anti-inflammatory drugs be used to treat osteoarthritis ? Rheum Clin North Am 1993; 19: 29-44.
6. Brandt KD. Should osteoarthritis be treated with nonsteroidal anti-inflammatory drugs? Rheum Clin North Am 1993; 19(3): 697-712.
7. Huskisson EC, Berry H, Gishen P, Jubb RW, Whitehead J. Effects of anti-inflammatory drugs on the progression of osteoarthritis of the knee. J Rheumatol 1995; 22: 1941-46.
8. Sheild MJ. Anti-inflammatory drugs and their effects on cartilage synthesis and renal function. Eur J Rheum Inflamm 1993; 13(1): 7-16.
9. Reichelt A, Forster KK, Fischer M, Rovati LC, Setnikar I. Efficacy and safety of intramuscular glucosamine sulphate in osteoarthritis of the knee. Arzneim Forsch 1994; 44: 75-80.
10. Tapadinhas MJ, Rivera IC, Bignamini AA. Oral glucosamine sulphate in the management of arthrosis: report on a multi-centre open investigation in Portugal. Pharmatherapeutica 1982; 3: 157-68.

11. Roden L. Effect of hexosamine on the synthesis of chondroitin sulphuric acid in vitro. Ark Kemi 1956; 10: 345-52.

12. Karzel K. Domenjoz R. Effects of hexosamine derivatives and uronic acid derivatives on glycosaminoglycane metabolism of fibroblast cultures. Pharmacology. 1971; 5: 337-45.

13. Vidal Y, Plana R, Bizarri D. Rovanti AL. Articular cartilage pharmacology. In vitro studies on GS and non steroidal antiinflammatory drugs. Pharmacol Res Comm 1978; 10: 557-69.

14. Vidal Y, Plana R, Karzel K. Glucosamine sulphate: its role in the articular metabolism. 2. Studies on rat and human articular cartilage. Fortschr Med 1980; 98: 801-06.

15. Bassleer C, Rovati L, Franchimont P. Stimulation of proteoglycan production by glucosamine sulphate in chondrocytes isolated from human osteoarthritic articular cartilage in vitro. Osteoarthritis Cart 1998; 6: 427-34.

16. Setnikar I, Cereda M, Pacini MA, Revel L. Antireactive properties of glucosamine sulphate. Arzneim Forsch 1991a; 41: 157-161.

17. Setnikar I, Pacini MA, Revel L. Antiarthritic effects of glucosamine sulphate studied in animal models. Arzneim Forsch 1991b; 41: 542-545.

18. Dijkgraaf LC, de Bont LGM, Boering G, Liem RSB. Normal cartilage structure, biochemistry, and metabolism: A review of the literature. J Oral Maxillofac Surg 1995; 53: 924-29.

19. Haskin CL, Milam SB, Cameron IL. Pathogenesis of degenerative joint disease in the human temporomandibular joint. Crit Rev Oral Biol Med 1995; 6: 248-277.

20. Westesson PL. Structural hard-tissue changes in the temporomandibular joints with internal derangement. Oral Surg Oral Med Oral Pathol 1985; 59:220-24.

21. Stegenga B, de Bont LG, Boering G. Osteoarthrosis as the cause of craniomandibular pain and dysfunction: a unifying concept. J Oral Maxillofac Surg 1989 47:249-56.

22. Nebbe B. Adolescent TMJ and craniofacial morphology. PhD thesis University of Alberta; Edmonton.1998;

23. Blaustein DI, Scapino RP. Remodeling of the temporomandibular joint disk and posterior attachment in disk displacement specimens in relation to glycosaminoglycan content. Plastic & Reconstructive Surg 1986; 78:756-64.

24. Nickel JC, McLachlan KR. In vitro measurement of the frictional properties of the temporomandibular joint disc. Archs Oral Biol 1994; 39: 323-31.

25. Stegenga B, de Bont LG, Boering G, van Willigen JD. Tissue responses to degenerative changes in the temporomandibular joint: a review. J Oral Maxillofac Surg 1991; 49:1079-88.

26. Tyler JA. Insulin-like growth factor 1 can decrease degradation and promote synthesis of proteoglycan in cartilage exposed to cytokines. Biochem J 1989; 260:543-8.

27. Israel HA, Saed-Nejad F, Ratcliffe A. Early diagnosis of osteoarthrosis of the temporomandibular joint: correlation between arthroscopic diagnosis and keratan sulfate levels in the synovial fluid. J Oral Maxillofac Surg 1991 49(7):708-11.

28. Israel HA, Diamond BE, Saed-Nejad F, Ratcliffe A. Correlation between arthroscopic diagnosis of osteoarthritis and synovitis of the human temporomandibular joint and keratan sulfate levels in the synovial fluid. J Oral Maxillofac Surg 1997; 55:210-7.

29. Shibata T, Murakami KI, Kubota E, Maeda H. Glycosaminoglycan components in temporomandibular joint synovial fluid as markers of joint pathology. J Oral Maxillofac Surg 1998 56:209-13.

30. Holmlund A, Hellsing G. Arthroscopy of the temporomandibular joint: occurrence and location of osteoarthrosis and synovitis in a patient material. Int J Oral Maxillofac Surg 1988; 17:36-40.

31. Holmlund A, Hellsing G, Axelsson S. The temporomandibular joint: a comparison of clinical and arthroscopic findings. J Prosth Dent. 1989; 62:61-5.

32. Israel HA, Diamond BE, Saed-Nejad F, Ratcliffe A. Correlation between arthroscopic diagnosis of osteoarthritis and synovitis of the human temporomandibular joint and keratan sulfate levels in the synovial fluid. J Oral Maxillofac Surg 1997; 55:210-7.

33. Israel HA, Diamond B, Saed-Nejad F, Ratcliffe A. Osteoarthritis and synovitis as major pathoses of the temporomandibular joint: comparison of clinical diagnosis with arthroscopic morphology. J Oral Maxillofac Surg 1998; 56:1023-7.

34. Dijkgraaf LC, Spijkervet FK, de Bont LG. Arthroscopic findings in osteoarthritic temporomandibular joints. J Oral Maxillofac Surg 1999; 57:255-68.

35. Van den Berg WB, van der Kraan PM, van Beuningen HM. Synovial mediators of cartilage damage and repair in OA. In: Brandt M, Doherty M, Lohmander LS, editors. Osteoarthritis. Oxford: Oxford Medical Publications; 1998: 157-67.

36. Merry P, Williams R, Cox N, King JB, Blake DR. Comparative study of intra-articular pressure dynamics in joints with acute traumatic and chronic inflammatory effusions: potential implications for hypoxic-reperfusion injury. Annals Rheumatic Diseases 1991; 50:917-20.

37. Allen RE. Blake DR. Nazhat NB. Jones P. Superoxide radical generation by inflamed human synovium after hypoxia. Lancet 1989; 2(8657):282-3.

38. Blake DR, Merry P, Unsworth J, Kidd BL, Outhwaite JM, Ballard R, Morris CJ, Gray L, Lunec J. Hypoxic-reperfusion injury in the inflamed human joint. Lancet 1989; 1(8633):289-93.

39. Vas AL. Double-blind clinical evaluation of the relative efficacy of ibuprofen and glucosamine sulphate in the management of osteoarthrosis of the knee in out-patients. Curr Med Opin 1980; 8: 145-149.

40. Muller-Fassbender H, Bach GL, Haase W, Rovati LC, Setnikar I. Glucosamine sulphate compared to ibuprofen in osteoarthritis of the knee. Osteoarthritis Cart 1994; 2: 61-69.

41. Qiu GX, Gao SN, Giacovelli G, Rovati L, Setnikar I. Efficacy and safety of glucosamine sulphate versus ibuprofen in patients with knee osteoarthritis. Arzneim-Forsch/Drug Res 1998; 48: 469-474.

42. Bradley JD, Brandt KD, Katz BP, Kalasinski LA, Ryan SI. Comparison of an antiinflammatory dose of ibuprofen, an analgesic dose of ibuprofen, and acetaminophen in the treatment of patients with osteoarthritis of the knee. New Eng J Med 1991; 325:87-91.

43. Bradley JD, Brandt KD, Katz BP, Kalasinski LA, Ryan SI. Treatment of knee osteoarthritis: relationship of clinical features of joint inflammation to the response to a nonsteroidal antiinflammatory drug or pure analgesic. J Rheumatol 1992; 19:1950-4.

44. Griffin MR, Brandt KD, Liang MH, Pincus T, Ray WA. Practical management of osteoarthritis. Integration of pharmacologic and nonpharmacologic measures. Arch Fam Med 1995; 4:1049-55.

45. Pinals RS. Pharmacologic treatment of osteoarthritis. Clin Therap 1992; 14:336-46.

46. Dieppe PA, Frankel SJ, Toth B. Is research into the treatment of osteoarthritis with non-steroidal anti-inflammatory drugs misdirected? Lancet 1993; 341(8841):353-4.

47. Bassleer C, Henrotin Y, Franchimont P. In-vitro evaluation of drugs proposed as chondroprotective agents. Int J Tiss Reac 1992; 14: 231-41.

48. Anderson MA. Oral chondroprotective agents. Part I: Common compounds. Compendium 1999; 21: 601-09.

49. Altman RD, Howell DS. Disease-modifying osteoarthritis drugs. In: Brandt KD, Doherty M, Lohmander LS, editors. Osteoarthritis. Oxford: Oxford University Press; 1998: 417-428.

APPENDICES

Appendix I

APOTEX INC.
CANADA'S PHARMACEUTICAL COMPANY
SOCIÉTÉ PHARMACEUTIQUE ENTIÈREMENT CANADIENNE

June 3, 1998

Dr. Norm Thie
TMD Investigational Unit
Faculty of Medicine & Oral Health Science
4068 Dentistry/Pharmacy Centre
University of Alberta
EDMONTON, ALBERTA
T6G 2N8

Via Courier

Dear Dr. Thie:

We are happy to donate to you as discussed, 6 kilograms of pure Ibuprofen USP for use in your study, *"Double-blind clinical evaluation of the effectiveness of Glucosamine Sulphate and Ibuprofen for treating patients with temporomandibular joint degenerative disease with pain"*. I have also enclosed a Certificate of Analysis of this lot of Ibuprofen for your records.

I wish you great success in your study and if you have any questions regarding this Ibuprofen, do not hesitate to call me directly at 416-401-7315.

Sincerely,
APOTEX INC.

Nicholas F. Cappuccino, Ph.D.
Vice-President Research & Development

NC/Donations/DrNThie/pl

cc: Dr. M. Spino
J. Kay

Scientific Affairs Fax # (416) 749-3234
150 Signet Drive, Weston, Ontario, Canada M9L 1T9
Tel: (416) 749-9300 • Telex: 065-27284 • Fax: (416) 749-9578

APOTEX INC.

CERTIFICATE OF ANALYSIS

QUALITY CONTROL LAB
RAW MATERIAL TESTING

Material Type : IBUPROFEN USP
Rec. No. : CD0518
Sample Type : RM-3090-05 ,Issue No. 11
Specification : 3090-05 ,Vers. No. 3

Storage Precautions : STORE IN TIGHT CONTAINERS

METHOD COMPONENT	SPECIFICATION	RESULT
APPEARANCE		
OVERALL APPEARANCE	FINE, WHITE, CRYSTALLINE POWDER.	PASS
PARTICLE SIZE (IMAGE)		
% UNDER 50 um	MINIMUM 90 %	98 %
% GREATER THAN 100 um	MAXIMUM 1 %	1 %
IDENTIFICATION		
IR SPECTRUM	CORRESPONDS TO STANDARD.	PASS
UV SPECTRUM	CORRESPONDS TO STANDARD.	PASS
% ABSORPTIVITY DIFF. 264 nm	MAXIMUM 3.0 %	0.8 %
% ABSORPTIVITY DIFF. 273 nm	MAXIMUM 3.0 %	1.2 %
HPLC: RETENTION TIME	CORRESPONDS TO STANDARD.	PASS
WATER	MAXIMUM 1.0 %	0.0 %
RESIDUE ON IGNITION	MAXIMUM 0.5 %	0.0 %
HEAVY METALS	NOT MORE THAN 0.002%.	LESS THAN 0.002%
ORGANIC VOLATILE IMPS		
BENZENE	MAXIMUM 100 PPM	NONE DETECTED
CHLOROFORM	MAXIMUM 50 PPM	NONE DETECTED
1,4-DIOXANE	MAXIMUM 100 PPM	NONE DETECTED
METHYLENE CHLORIDE	MAXIMUM 500 PPM	NONE DETECTED
TRICHLOROETHYLENE	MAXIMUM 100 PPM	NONE DETECTED
SPECIFIC RELATED COMPOUND		
4-ISOBUTYLACETOPHENONE	MAXIMUM 0.1 %	0.00 %
RELATED COMPOUNDS		
IB RC7	MAXIMUM 0.3 %	0.03 %
IB RC6	MAXIMUM 0.3 %	NONE DETECTED
IB RC5	MAXIMUM 0.3 %	0.07 %
MAX. UNIDENTIFIED COMPOUND	MAXIMUM 0.3 %	TRACE
TOTAL RELATED COMPOUNDS	MAXIMUM 1.0 %	0.10 %

DATE PRINTED : 5/29/98 18:20:40
(200007468 / 100006844)

APOTEX INC.

CERTIFICATE OF ANALYSIS

QUALITY CONTROL LAB
RAW MATERIAL TESTING

Material Type : IBUPROFEN USP
Rec. No. : CD0518
Sample Type : RM-3090-05 ,Issue No. 11
Specification : 3090-05 ,Vers. No. 3

Storage Precautions : STORE IN TIGHT CONTAINERS

METHOD COMPONENT	SPECIFICATION	RESULT
ASSAY		
AVG. ASSAY (anhydrous basis)	97.0 to 103.0 %	100.6 %

Expiry Date: 31-MAY-00

Released By: V Subramaniam. Date: 29-MAY-98 17:54

V. Subramaniam
Supervisor, Q.C. Raw Materials Testing

DATE PRINTED : 5/29/98 18:20:40
(200007468 / 100006844)

Jamieson
Since 1922

From the Desk of:

Stanley M. Chacko
Quality Assurance Coordinator
Phone: (519) 974-8482 Ext. 292
Fax: (519) 974-4742
E-mail: schacko@jamiesonvitamins.com

[redacted]

TO:	COMPANY:
Dr. Norman Thie	University of Alberta
FAX NUMBER:	DATE:
(403) 492-1624	08/27/98
PHONE NUMBER:	TOTAL NO. OF PAGES INCLUDING COVER:
(403) 492-2101	4
RE:	CC:
Glucosamine Sulfate C. of A.	Daniel Houde, Manager of Technical Support

☐ URGENT ☐ FOR REVIEW ☐ PLEASE COMMENT ☐ PLEASE REPLY ☐ PLEASE RECYCLE

Dear Dr. Thie,

I apologize for the delay in sending you the Certificate of Analysis for the Glucosamine Sulfate capsules. I have also enclosed our Ingredient Listing for this product.

If there is anything else you require, please contact me.

Thank you,

Best Regards,

Stan Chacko

QUALITY ASSURANCE

REQUEST FOR SAMPLE ANALYSES

SUBMITTED TO: Quality Control **DATE:** 08/11/98

SUBMITTED BY: Stan Chacko

PRODUCT: Glucosamine Sulfate Capsules	**VENDOR:** C.E. Jamieson
PRODUCT CODE: F2086	**VENDOR LOT #:** 28687

ANALYSES REQUESTED:

		RESULT	ANALYST
1.	Glucosamine Sulfate potency per capsule	467.2 mg/caps.	FX-1-102
2.	Microbiological Assays:		
3.	Total Plate Count	10 bacteria/g	DR-M48-178
4.	Yeast and Mould Count	<10 organisms/g	DR-M48-178
5.	*Escherichia coli*	Absent	DR-M48-178
6.	*Salmonella* Species	Absent	DR-M48-178
7.	*Staphylococcus aureus*	Absent	DR-M48-178
8.	*Pseudomonas aeruginosa*	Absent	DR-M48-178
9.			
10.			

REMARKS:

Purity, Potency, and Identity of Glucosamine Sulfate per Dose Required.
Microbiological Assays
Manufacturing Lot # 18583
Food product

DATE COMPLETED: 08/11/98 **QUALITY ASSURANCE:**

Jamieson
Since 1922

Jamieson
Since 1922

CERTIFICATE OF ANALYSIS

GLUCOSAMINE SULFATE

TESTS	SPECIFICATIONS	RESULTS
DESCRIPTION	WHITE OR PALE BROWN CRYSTALLINE POWDER OR GRANULAR CRYSTALS	CONFORMS
OPTICAL ROTATION	+52.0 TO +56.0°	+53.2°
pH	4.0-4.4	4.3
TRANSMITTANCE	>=96.0%	97.9%
CHLORIDE (Cl)	11.9 - 12.8%	12.3%
IRON (Fe)	<=10 PPM	CONFORMS
ARSENIC	<=1 PPM	CONFORMS
HEAVY METALS (Pb)	<=10 PPM	CONFORMS
LOSS ON DRYING	<=0.5%	0.11%
RESIDUE ON IGNITION	23.8 - 25.7%	24.7%
ASSAY	96.0 - 104.0%	101.1%

APPENDIX II.

Pressure Pain Threshold (&SD) values (kPa)[40] converted to kg/cm^2 and used as cut off for the present study.

	Males			Females		
	Total (right and left)			Total (right and left)		
Muscle	Mean	SD	Kg/cm^2	Mean	SD	Kg/cm^2
Anterior temporalis	308.0	93.34	2.19	239.1	58.5	1.84
Middle temporalis	357.8	134.9	2.27	274.6	60.9	2.18
Posterior temporalis	354.2	109.3	2.50	298.0	76.4	2.26
Deep masseter	249.2	70.6	1.82	201.4	50.3	1.54
Anterior masseter	228.1	41.5	1.90	188.0	39.3	1.51
Inferior masseter	219.0	58.0	1.64	168.2	30.3	1.41
Medial pterygoid	156.9	30.1	1.29	133.5	30.4	1.05

APPENDIX III.

Intra-rater reliability of measurement tools for all variables - 5 TMD patients independent of study over 3 consecutive days (Mon., Wed., Fri.)

Variable	Reliability Coefficient
1) Functional pain evaluation (CAS)	
a) Pain when yawning	0.8923
b) Pain when chewing	0.8615
c) Pain when talking	0.9747
d) Pain when laughing	0.9099
2) Pain free mouth opening (mm)	0.9666
3) Voluntary mouth opening (mm)	0.9336
4) Muscle palpation	
i) Right	
a) Anterior temporalis	0.8174
b) Middle temporalis	0.8577
c) Posterior temporalis	0.8087
d) Anterior masseter	0.9546
e) Inferior masseter	0.9424
f) Deep masseter	0.9098
g) Medial pterygoid	0.9137
ii) Left	
a) Anterior temporalis	0.9791
b) Middle temporalis	0.9175
c) Posterior temporalis	0.9667
d) Anterior masseter	0.9620
e) Inferior masseter	0.8873
f) Deep masseter	0.7954
g) Medial pterygoid	0.9609

APPENDIX IV.

Pretreatment comparison of study groups.

Variable	Glucosamine sulphate n=21	Ibuprofen n=18	Observed mean difference between treatments	p
Demographics				
1) Age (years)	36.62(10.30)	38.73(13.30)	-2.11	0.55
2) Disease duration (months)	16.61(8.06)	15.09(8.01)	1.52	0.53
Measured Variables				
1) Functional pain evaluation (CAS)	23.18(6.53)	19.52(6.74)	3.66	0.09
2) Pain free mouth opening (mm)	24.71(9.25)	26.06(8.12)	-1.34	0.64
3) Voluntary mouth opening (mm)	34.52(7.26)	37.39(7.46)	-2.87	0.23
4) BPI questionnaire (CAS)				
i) Pain intensity	22.67(5.34)	19.36(7.65)	3.31	0.13
ii) Pain interference (CAS)	32.26(13.26)	25.19(14.79)	7.07	0.12
5) Extra oral masticatory muscle pain (positive on 14 sites)	7.81(5.07)	8.00(5.43)	-0.19	0.91

APPENDIX V

Sample size estimation

Sample size estimation was based on the two published studies on humans comparing Ibuprofen and GS (39,40).

To determine sample size we must specify

1) σ^2;
2) The probability, α, of a Type I error;
3) The magnitude of the difference $\mu_1-\mu_2$ to be detected; and
4) The power, $1-\beta$, or equivalently the probability of a Type II error, β.

$$\Delta = \frac{|\mu_1-\mu_2|}{\sigma}$$

$\Delta = 2/4.66$

$\Delta = 0.4292$

$$n = \frac{2(Z_{1-\alpha/2} + Z_{1-\beta})^2}{\Delta^2}$$

$$n = \frac{2(1.645+0.84)^2}{0.4292^2}$$

$n = 67$

Power = 0.80

Therefore, 67 patients are needed per treatment group for a total of 134 in this investigation.